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METABOLIC ASPECTS OF HIBERNATION IN THE HEDGEHOG (*ERINACEUS EUROPÆUS* LINNÆUS 1758)

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METABOLIC ASPECTS OF

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(*ERINACEUS EUROPAEUS* LINNAEUS 1758)

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Hwi sofwen j så sött i syndsens dwala ?
Lybecker (c. 1715)

Those who suffer from insomnia or cold feet may view with envy the many creatures who dream away the snowy months.
Barker (1958)

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immobiles quasi mortui jacent, sed estivo tempore reviviscunt
De Beauvais V. (1470-71)

This thesis is based on the following papers:

I. Olsson Sven-Olle R. (1972) Dehydrogenases (LDH, MDH, G-6-PDH and alpha-GPDH) in the heart, liver, white and brown fat. In Seasonal variations in the physiology and biochemistry of the european hedgehog (Erinaceus europaeus) including comparisons with non-hibernators, guinea-pig and man. (Edited by Johansson B.W. and Senturia J.B.) Acta physiol. scand. Suppl. 380, 62-95.

II. Olsson Sven-Olle R. (1972) Ultrastructure of brown adipose tissue and myocardium. Ibidem. 117-130.

III Olsson Sven-Olle R. (1974) Comparative studies on the temperature dependence of Lactic and Malic dehydrogenase from a homeotherm, guinea pig (Cavia porcellus), two hibernators, hedgehog (Erinaceus europaeus) and bat (Nyctalus noctula); and two poikilotherms, frog (Rana temporaria) and cod (Gadus callarias). In manuscript. Submitted to Comp. Biochem. Physiol.

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Abbrevations.

E_a	Energy of activation in kJ/mole (Precht *et al.*, 1973)
E_i	Energy of inactivation in kJ/mole (Precht *et al.*, 1973)
alpha-GPDH	alpha-Glycerophosphate dehydrogenase (EC. 1.1.1.8.)
G-6-PDH	Glucose-6-phosphate dehydrogenase (EC. 1.1.1.49.)
LDH	Lactic dehydrogenase (EC. 1.1.1.27.)
LDH 1,2,3,4,5	The isoenzymes of LDH in decreasing order according to anodal mobility.
LDH-H (B)	The heart type of LDH, consisting of 4 H (B)-monomers.
LDH-M (A)	The skeletal muscle type of LDH, consisting of 4 M (A)-monomers.
MDH	Malic dehydrogenase (EC. 1.1.1.37.)
C-MDH	Cytoplasmatic MDH
M-MDH	Mitochondrial MDH
RQ	Respiratory quotient: expired CO_2/inspired O_2.
Q_{10}	The ratio between two activities at 10°C temperature interval.

Introduction.

The fact that some animals retire and enter into a state of dormancy at autumn has been known for a long time. "Aristoteles sagt die Thiere verbergen sich, um sich sowohl gegen die grosse Kälte, als gegen die zu grosse Wärme zu schützen" (Barkow 1846). This work "Der Winterschlaf" by H.C.L. Barkow, Berlin 1846, was the first compilation of observations on hibernation in the animal kingdom. References to publications on hibernation, which appeared between 1846 and 1956 are found in Kalabuchov (1956).

Modern physiological research on hibernation began with the publication of the following important books:

(1). Nikolaj Ivanovitj Kalabuchov "Spjatjka Zjivotnych", (Hibernation of animals) first edition 1936 and third edition 1956, Gorki State University Press, Charkov.
(2). Benedict F.G. and Lee R.C. Hibernation and marmot physiology, Carnegie Institution of Washington, Publ. 497, 1938, Waverly Press, Baltimore.
(3). Martin Eisentraut Der Winterschlaf mit seinen ökologischen und physiologischen Begleiterscheinungen, 1956, Veb. Gustav Fischer Verlag, Jena.
(4). Charles Kayser The Physiology of Natural Hibernation, 1961, Pergamon Press, Oxford.

The value of the collaboration between scientists through H. I. E. (Hibernation Information Exchange) and the arrangements of the four Hibernation Symposia cannot be overemphasized for the progress of the hibernation research. The four symposia are published in the following books:

(1). Mammalian Hibernation I Edited by Lyman C.P. and Dawe A.R. Bull. Mus. Comp. Zool. (Harv.) 124, 1960.
(2). Mammalian Hibernation II, Edited by Suomalainen P. Ann. Acad. Sci. Fenn. Ser. A. IV, 71, 1964.
(3). Mammalian Hibernation III Edited by Fisher K.C., Dawe A.R., Lyman C.P., Schönbaum E., South F.E., Oliver & Boyd, Edinburgh 1967.
(4). Hibernation - Hypothermia, Perspectives and Challenges. Edited by South F.E., Hannon J.P., Willis J.R., Pengelley E.T., Alpert N.R., Elsevier, Amsterdam 1972.

Another four books, which are important for hibernation research are:

(1). Adaptation to the environment Edited by Dill D.E. Handbook of Physiology Section 4. American Physiological Society Washington D.C. 1964.
(2). Precht H., Cristophersen J., Hensel H. and Larcher W. Temperature and Life. Springer Verlag Berlin 1973.
(3). Depressed Metabolism Edited by Musacchia X.J. and Saunders J.F. Elsevier, New York 1969.
(4). Mrosovsky N. Hibernation and the Hypothalamus. Appleton, New York 1971.

During autumn and winter terrestrial homeothermic animals are met with several difficulties:

(1). the temperature decreases
(2). the day shortens and
(3). the access or supply of food decreases.

Homeothermic animals have adapted to these changes in many ways:

(1). By hibernating. Hibernation is generally restricted to three Mammalian orders: Insectivora, Chiroptera and Rodentia, but there are reports that some birds may hibernate (Phalaenoptilus nuttallii) and that Apidae and Trichilidae show cold lethargy (Dorst 1962).
(2). By migration. Migration shows many physiological resemblances to hibernation. Birds and bats (Chiroptera) are the most well known representatives of this group. (Dorst 1962, Griffin 1970)
(3). By physiological and ecological adaptations. Examples of these adaptations are increased insulation by winter fur (Irving 1964) and hiding below the snow or below the ground surface.

Before mammalian hibernation is treated in more detail, some basic facts about the temperature responses in the animal kingdom must be remembered. Animals can be divided into two groups poikilotherms (their body temperature changes in the same direction as the external temperature, Jankowsky 1973) and homeotherms (they maintain a constant body temperature, true homeotherms are birds and mammals). The old terms "cold blooded" and "warm blooded" animals are not quite correct as some "cold blooded" animals may occasionally attain a higher body temperature i.e. the desert iguana Dipsosaurus dorsalis 42°C (Norris 1953, Kemp 1969

Besides, there are many examples of thermoregulation in poikilotherms i.e. by behavioural means, by absorption of heat radiation, by evaporation of water, by counter current heat exchanges and by spasmodic muscular contraction (Jankowsky 1973). But, endothermic metabolic regulation of body temperature of poikilotherms has only been reported from some large lizards (Varanus varanus) and snakes (Jankowsky 1973). However, most poikilotherms are ectothermic, "their body temperatures are independent of the heat produced by their oxidative metabolism and are determined exclusively by heat acquired from the environment" (Gordon et al., 1972). Endothermic are animals (homeotherms, mammals and birds), which produce sufficient heat by their own oxidative metabolism to maintain their body temperatures (Gordon et al., 1972). Besides, poikilotherms are bradymetabolic (slow metabolism) in comparison with homeotherms, which are tachymetabolic (fast metabolism) (Hensel et al., 1973).

The size of the body plays a great role for the different ways of thermoregulation i.e. the small black insects can very easily absorb heat because their great surface/volume ratio and the big reptiles can use endothermic metabolic heat because of their small surface/volume ratio (Jankowsky 1973). The homeotherms, however, are extremely dependent on this surface/volume ratio, since they have to maintain a constant high body temperature. Kleiber (1947) expressed this dependence in the formula:

$\log M = 1{,}83 - 0{,}756 \log W \pm 0{,}05$ (M = heat formation/unit time, W = body weight).

A very small mammal the shrew Sorex cinereus has a metabolic rate, which is 65 times that of man and 200 times that of elephant. Besides, the body weight of the shrew (2,5g) seems to be near the lower limit for mammals since at still lower sizes the uptake of nutrition could not cover the extremely high energy demands (Hensel et al., 1973). The lowest body weights found among the birds are 1,6 - 1,8 g (some species of kolibris) (Ulfstrand 1963). From this point of view it is obvious that small homeotherms have great difficulties in getting enough food, especially when the energy demand is increased at decreased environmental temperatures. In this situation a poikilotherm passively decreases the body temperature and thus saves energy. Among some very small homeotherms, kolibris and bats, the body temperature decreases during the resting period of day. This is quite essential for the small kolibris and bats. This phenomenon is called "Tagesschlaflethargie" among the bats (Eisentraut 1956) and may be regarded as an adaptation to save energy.

Hibernation of mammals is an adaptation to the seasonal changes in the environment. The best definition of hibernation is published by Bartholomew and Hudson (1960): "A regulated, periodic phenomenon in which body temperatures become readjusted to new, lower levels approximating ambient, and heart rate, metabolic rate and other physiologic functions show corresponding reductions from which spontaneous or induced arousal to normal levels is possible at all times".

Hibernation is an adaptation which requires:

(1). survival and maintenance of the coordinated physiological activity of the organism and particularly the cardiac and nervous activity at temperatures near $0^{o}C$,

(2). endogenous seasonal changes of physiological parameters, these changes are reflections of seasonal changes in the environment.

1. The increased cold tolerance of the tissues from hibernators, compared to non-hibernators.

It is known that diverse tissues from hibernators have a higher degree of anoxic (Hiestand et al., 1950, Biörck et al., 1956, Covino and Hannon 1959, Hannon et al., 1961, Kayser and Malan 1963, Andjus et al., 1964) and cold tolerance (Hannon et al., 1972) than other homeotherms. The best known tissue in this respect is the myocardium. Johansson (1967) reviewed the results obtained from in vitro and in vivo experiments from hibernating and non-hibernating hibernators and non-hibernators. To summarize the results, there is a great difference between the cold tolerance of the coordinated activity of the myocardium from hibernators and non-hibernators, which is independent of whether the animal is hibernating or not. This inborn difference in cold resistance between hibernators and non-hibernators may depend on:

(1). differences in the composition of the cell membranes (Tait 1922, Huttonen and Johansson 1963, McMurchie 1973)

(2). differences in the innervation of the myocardium (Nielsen 1968)

(3). differences in the cold response of the intermediary metabolism. The metabolic differences (1) between hibernating and non-hibernating hibernators and (2) between hibernator and non-hibernator obtained by South (1958, 1960 a and b) showed the highest E_a (energy of activation) of O_2-consumption from tissues of non-hibernators and lowest E_a from tissues of hibernating hibernators. The same differences in E_a of anaerobic glycolysis of brain slices between non-hibernator and hibernator were obtained by Burlington and

Wiebers (1967). Previously Vroman and Brown (1963) have shown differences in E_a of succinic dehydrogenase between rat (52,3 kJ/mole) and frog (18,4 kJ/mole). Woodard and Zimny (1973) found a lower E_a for the same enzyme from kidney but not from the heart or brain of hibernating ground squirrels (Citellus tridecemlineatus) compared with that of non-hibernating animals. These results suggest that some other enzyme in the oxidation of malate + pyruvate (South 1960) and glucose (South 1958) must be responsible for the difference in E_a observed by South (1958 and 1960) and Burlington and Wiebers (1967). Because of this background, it was necessary to study the temperature dependence of the two important enzymes Lactic dehydrogenase (LDH) (EC. 1.1.1.27.) and Malic dehydrogenase (MDH) (EC.1.1.1.37.) from homeotherms (guinea pigs), hibernators (hibernating and non-hibernating hedgehogs and hibernating bats), and poikilotherms (frogs and cod) (Paper III).

2. The seasonal cycle of hibernators.

This cycle may be divided into the following periods:

(1). The summer period, is characterized by a normal homeothermic response to environmental changes. This is true for all mammalian hibernators except bats, which show "Tagesschlaflethargie" (Eisentraut 1956). The summer period consists of a reproductive period and a preparative period for hibernation.

(2). The autumnal period (prehibernation) is characterized by the fattening of the animals and gradual endocrine changes.

(3). The proper entry into hibernation is a transition from the active state into the depressed metabolic state of the low body temperature, which is called

(4). hibernation. During the period of hibernation the animals are not in a continous hibernation, but wake up at a certain degree of periodicity (Kristoffersson and Soivio 1964).

(5). The arousal from hibernation can be initiated by many kinds of stimuli and is characterized by a thermogenesis of the brown adipose tissue and a concentration of the blood flow to the fore part of the body (Hayward and Lyman 1967, Lyman and Chatfield 1950, Lyman 1970, Studier 1974).

The literature about hibernation is vast but the basic mechanisms involved are still not understood. First it is essential to remember that hibernation is represented in different mammalian orders: Chiroptera (Rhinolophidae and Vespertilionidae), Insectivora and Rodentia. Hibernation is most probably a secondary adaptation of homeothermic mammals and not a primitive characteristic (Cade 1964). Thus there may be great differences

between the seasonal physiological changes in hibernators of different species. The golden hamster (Mesocricetus auratus) needs a long adaptive period with food and water, before the animal enters into hibernation (Smit-Vis and Smit 1963). The bats, however, are very apt to enter into hibernation.

The metabolic seasonal changes in hibernators are reviewed by South and House (1967) and Burlington (1972). It has been known for a long time that RQ (Respiratory quotient CO_2/O_2) is about 0,7 during hibernation, which indicates fat catabolism (South and House 1967). Several results indicate that the lipogenesis is decreased during hibernation (Denyes and Carter 1961, Whitten and Klain 1969, Klain and Rogers 1970, Tashima et al., 1970). Protein synthesis has also been found to be reduced during hibernation (Manasek et al., 1965, Whitten and Klain 1968). The metabolism of carbohydrates has some interesting aspects. The level of blood glucose during hibernation seems to be dependent on whether the animal eats during the short awake periods or not (Burlington 1972). In golden hamsters hypoglycemia has not been found (Lyman and Leduc 1953, Helle 1953). Hamsters do eat during the short awake periods of the hibernation. (pers. obs.).

Glycogenolysis and gluconeogenesis are the major possible sources of glucose during hibernation. Some workers show a decrease of liver glycogen (Burlington and Klain 1967, Galster and Morrison 1970, Tashima et al., 1970), but according to Tashima et al., the decrease of liver glycogen could not maintain the plasma glucose level for the hibernating period. Thus gluconeogenesis must be important during hibernation for the maintenance of the plasma glucose level (Burlington 1972, Burlington and Klain 1967 and Klain and Whitten 1968).

During arousals from hibernation RQ increases to about 1,0, which is thought to reflect an increased oxidative metabolism of glucose (South and House 1967). But according to Burlington (1972) gluconeogenesis may also be important for the generation of glucose for arousal, but increased catabolism of lipids and proteins is also suggested during arousal. To contribute to the knowledge of seasonal changes of intermediate metabolism in hibernators four key enzymes were selected: LDH (EC. 1.1.1.27.) MDH (EC. 1.1.1.37.), G-6-PDH (EC. 1.1.1.49.) and alpha-GPDH (EC. 1.1.1.8.) as indicators of changes in metabolic pathways in different organs: heart, liver, white and brown fat. In these studies a hibernator, the hedgehog, was compared with the guinea pig. (Paper I.).

Seasonal changes in brown adipose tissue of hibernators have been discussed by Smalley and Dryer 1967). With the changes of enzyme activities and protein concentration as a background, it was logical to correlate these results with the ultrastructural seasonal changes of brown adipose tissue in hedgehog. Besides, since there has been dispute about the seasonal ultrastructural changes in the heart of the hibernator (Poche 1959, Moreland 1962, Zimny and Moreland 1968, Rosenquist and Zimny 1969, Rosenquist 1970, Aloia et al., 1971, Burlington et al., 1972) it was essential to see if there are any obvious ultrastructural seasonal changes in the myocardium of the hedgehog compared to guinea pig. (Paper II.).

Materials and Methods.

The experimental animals and the methods used have been described in detail in papers I, II and III.

Note:

The pH dependence of the MDH activity from hibernating hedgehogs was examined in glycine-buffer according to Clark (1928). pH was always measured at 24°C both before and after the reaction had taken place. The pH of the actual reaction temperature was extrapolated from Clark (1928).

Results.

The results presented in paper I show:

1. There are seasonal changes in the activities of LDH, MDH, G-6-PDH and alpha-GPDH from heart, liver, white and brown fat of both hedgehog and guinea pig. In all organs from hedgehog except white fat, brown fat (G-6-PDH and alpha-GPDH) and liver (alpha-GPDH) all enzymes have maximal activity during hibernation. White fat LDH, MDH and alpha-GPDH show an increase in activity between hibernation and spring. However, G-6-PDH from white fat decreased before hibernation. The changes of G-6-PDH and alpha-GPDH from brown fat and alpha-GPDH from liver have the same principal pattern of variation in activity as those from white fat.

   The changes in enzymatic activity from guinea pig organs are quite different from those of hedgehog. No significant changes have been found from the enzyme activities of white adipose tissue or for G-6-PDH activity from all three tissues of guinea pig. Both LDH and MDH activity appear to be minimal at summer and maximal at autumn and spring. The changes in alpha-GPDH activity in heart and liver are similar, with maximal values at spring and minimal values at autumn.

2. The activities of LDH and alpha-GPDH in heart and G-6-PDH in liver from hedgehog are significantly higher than the corresponding activities of guinea pig. On the other hand the activities of MDH and G-6-PDH in heart, alpha-GPDH in liver and MDH, G-6-PDH and alpha-GPDH in white fat from guinea pig are significantly higher than the activities from hedgehog.

3. A seasonal variation of both the LDH-H activity and the energy of activation (E_a) of heart LDH from hedgehogs has been found. The variations are small, but the decrease of the energy of activation between winter and spring and the decrease of LDH-H between autumn and winter are significant.

In paper III a positive correlation ($0{,}001<P<0{,}01$) between LDH-H and E_a of LDH and a negative correlation ($0{,}001<P<0{,}01$) between LDH-H and E_i of LDH have been described from hearts, livers, brains and skeletal muscles from

hedgehogs, guinea pigs and bats (Nyctalus). The different values for E_a of LDH obtained from different tissues were a consequence of different isoenzyme distribution (i.e. different LDH-H/LDH-M ratios). No correlation ($P>0,05$) was found between C-MDH and E_a of MDH. Furthermore the results from paper I were studied in paper III and a correlation between LDH-H and E_a of hedgehog heart LDH was established ($0,01<P<0,05$).

The energy of activation of both LDH and MDH do not differ from different species (homeotherm: guinea pig, hibernators: hedgehog and bat, poikilotherms: frog and cod) and the Arrhenius plots were linear in a long temperature interval (paper III), but the temperature maxima of MDH activity differ from poikilotherms and homeotherms and the Arrhenius plots were linear between 0 - 40°C for the mammals and between 0 - 30°C for the poikilotherms (paper III). The pH optima of MDH from hibernating hedgehog have been found to vary with temperature. The pH optima were found to be 8,6 ($pH_{47°C}$), 9,6 ($pH_{34°C}$) and 10,4 ($pH_{9°C}$). When these pH-values were measured at 24°C they were found to be 9,1 (47°C), 9,8 (34°C) and 10,0 (9°C).

An interesting observation is the difference in anodal electrophoretic mobility of C-MDH between guinea pig, hedgehog, bat and frog (paper III). The LDH isoenzymes from these animals showed the following interesting facts:

1. a relatively high percentage of LDH-H in bat pectoralis muscle, hedgehog brown adipose tissue and guinea pig liver and kidney.
2. a total loss of LDH_2 in frog heart and the presence of LDH_6 in bat liver. (paper III).

Seasonal variations in the ultrastructure of brown adipose tissue of hedgehogs (Paper II) showed a greater number of mitochondria and fat droplets during hibernation compared with the non-hibernating state (summer). In the summer the fat droplets are much bigger than in the winter and thus the fat content may be higher during the summer. The brown adipocyte from spring hedgehogs had about the same ultrastructural appearance as the brown adipocyte from the hibernating hedgehogs (paper II). No ultrastructural changes or differences were found in the myocardium of hedgehogs or guinea pigs (paper II).

Discussion.

The changes in enzyme and isoenzyme activities described in paper I suggest the following seasonal changes in the metabolic capacity of the hibernator, hedgehog:

1. A shift towards more anaerobic metabolism during hibernation.

This suggestion is based on the significant decrease of LDH-H in hedgehog heart between autumn and winter and the small insignificant tendency to increase between spring and summer. The same results with myocardium have been obtained by Burlington and Sampson (1968), who worked with the ground squirrel Citellus tridecemlineatus. Brush (1968), studied the LDH isoenzymes of hibernating and non-hibernating bats (Eptesicus fuscus) and found a shift in LDH isoenzymes of nonflight skeletal muscles towards increased proportions of LDH-M. Thus it seems as if LDH isoenzymes have a certain tendency to shift the distribution from LDH-H towards LDH-M during hibernation. However, in the non-hibernator, the rat, Blatt et al., (1965) found a decrease of LDH-M at cold adaptation. This shift of the LDH-isoenzyme distribution presumably depends on functional differences between LDH-H and LDH-M. The theory of Pfleiderer and Wachsmuth (1961) and Cahn et al., (1962), which suggests (1) that aerobic conditions in the cell favor the synthesis of LDH-H and anaerobic conditions favor the synthesis of LDH-M and (2) that high pyruvate concentrations inhibit more LDH-H than LDH-M. This will favor the pyruvate reduction in anaerobic cells which is discussed both in paper I and III. In the latter paper the temperature dependence of this pyruvate inhibition is treated. In a recent review of LDH by Everse and Kaplan (1973) the problem of substrate inhibition and aerobic contra anaerobic metabolism of LDH-H and LDH-M is discussed and rather convincing experimental data are presented which support the theory that LDH-M functions as pyruvate reductase to provide for the reoxidation of NADH from glyceraldehyde dehydrogenase. LDH-H, however, forms an abortive complex, consisting of enzyme, NAD and pyruvate at lower pyruvate concentrations than LDH-M. The effect of this abortive complex is the maintenance of a constant NAD/NADH ratio. An increase in NADH will reactivate the enzyme, resulting in the oxidation of some of the reduced coenzyme, whereas a significant decrease in the NADH level will promote abortive complex formation and inhibit the oxidation of NADH. In the same way an increase of the pyruvate level will promote the formation of the complex, resulting in inhibition of pyruvate reduction and pyruvate is kept available for the entrance into the Krebs´cycle. The abortive complex is rapidly dissociated by lactate, which implies that the oxidation of lactate is not

influenced by the complex. Everse and Kaplan (1973) believe it is likely that LDH-H may be one of the most important enzymes in the energy producing pathway of aerobic tissues, since it may exert a regulatory effect on the operation of the Krebs´cycle as well as on the electron transport pathway. Besides, it seems likely LDH-H is an enzyme that is under metabolic control and is regulated by its own oxidized product as well as the oxidized coenzyme (Everse and Kaplan 1973). The objections, which have been raised to this theory of Pfleiderer and Wachsmuth (1961) and Cahn et al., (1962) by Vesell and Pool (1966) Wuntch et al., (1969, 1970 a & b) are based on the fact that pyruvate inhibition occurs at unphysiological concentrations. Everse and Kaplan (1973) reject their criticism by pointing to the fact that no abortive complex can be formed during the short time used and at the low NAD concentrations used by Vesell and his co-workers. It is not possible to penetrate all the evidences and indices here, but it seems as if the theory of Pfleiderer and Wachsmuth (1961) and Cahn et al., (1962) may be valid. (See also Cahn 1963, 1964, Goodfriend and Kaplan 1963, Wilson et al., 1963, 1964, Dawson et al., 1964, Kaplan 1964, Salthe 1965, Lindy and Rajasalmi 1966, Altman and Robin 1969, Blix and From 1971 and Peter et al., 1971).

The quotient LDH/MDH, which was proposed by Bücher and Klingenberg (1958) may give some informations about aerobic versus anaerobic metabolism. As discussed in paper I no changes in the quotient can be observed during the four seasons, but liver of hedgehog has a much higher quotient than liver of quinea pig, which suggests a more anaerobic metabolism in hedgehog liver than in guinea pig liver. This is also supported by the high percentage of LDH-H in guinea pig liver in comparison with hedgehog liver (paper III). It has been stated that hibernators are more capable of anaerobic metabolism than non-hibernators (Hiestand et al., 1950, Kayser and Malan 1963, Andjus et al., 1964, Covino and Hannon 1959, Hannon et al., 1961, Biörck et al., 1956) and that the capacity for anaerobic glycolysis in the myocardium is increased during hibernation (Burlington and Wiebers 1966). The present study lends support to this opinion.

2. An increased capacity for gluconeogenesis during hibernation.

This very tentative suggestion is partly based on the high activity level of MDH in heart and liver during hibernation of hedgehog. The high MDH activity during hibernation may also be an expression of a compensatory mechanism to preserve the aerobic metabolism of Krebs´cycle. But it is also necessary to remember that MDH in some cases may serve as a NADH oxidizing enzyme in the same way as LDH and alpha-GPDH at anaerobiosis (Chappell 1968). With the above

discussion about anaerobic metabolism as a background it is essential to discuss this possibility of gluconeogenesis. As is discussed in paper I MDH may play an important role in the gluconeogenesis, since both a mitochondrial (M-MDH) and a cytoplasmatic part (C-MDH) exist. Two important steps in the gluconeogenesis are the intramitochondrial formation of oxaloacetate from pyruvate by pyruvate carboxylase (EC. 6.4.1.1.) and the extramitochondrial formation of phosphoenolpyruvate from oxaloacetate by phosphoenolpyruvate carboxykinase (EC. 4.1.1.32.) (Hanson and Garber 1972). Oxaloacetate is practically impermeable to the mitochondrial membrane and thus oxaloacetate must be reduced by M-MDH to malate, which can penetrate the mitochondrial membrane. Fahier and Strmecki (1969) suggested from their bovine liver and heart MDH experiments that M-MDH from liver is better suited for oxaloacetate reduction than M-MDH from heart. Then, malate is oxidized by C-MDH to oxaloacetate in the cytoplasme. This reaction and the oxidation of lactate give NADH, which is necessary for glyceraldehyde-3-phosphatedehydrogenase. Oxaloacetate may also be transaminated to aspartate which is permeable to the mitochondrial membrane. Thus MDH may play an important part in the gluconeogenic process. This model is applicable to rat, mouse and hamster liver, where phosphoenolpyruvate carboxykinase is cytosolic (Nordlie and Landy 1963, Ballard and Hanson 1967), but in most animals there are both cytosolic and mitochondrial phosphoenolpyruvate carboxykinase (Garber et al., 1972). In rabbit and guinea pig livers Arinze et al., (1973) found that half of the gluconeogenic flux went through the mitochondrial form of phosphoenolpyruvate carboxykinase. There are still many questions to be answered before the role of the mitochondrial form is evaluated (Arinze et al., 1973) No studies have been done to evaluate these complex metabolic interactions in gluconeogenesis at hibernation.

The hedgehogs in this study showed a decrease in blood glucose but an increase in both heart and liver glycogen during hibernation (Thorell et al., 1972). This points to an increased gluconeogenesis during hibernation, which is supported by the work of Burlington and Klain (1967), Whitten and Klain (1968) and Klain and Whitten (1968). Hibernating animals are known to oxidize fatty acids (RQ=0,7) (South and House 1967) and fatty acid oxidation stimulates gluconeogenesis either through an acetyl-CoA dependent activation of pyruvate carboxylase or to an increased supply of reducing equivalents within the mitochondria (Scrutton and Utter 1968). Thus there is considerable evidence for increased gluconeogenesis during hibernation.

3. A high dependence in the liver and perhaps also in the heart of hibernators on the pentose-shunt especially during hibernation.

The G-6-PDH activity in liver of hedgehog is about twice that of guinea pig. Further G-6-PDH activity decreases after hibernation both in the liver and in the heart. The essential effect of G-6-PDH is the production of NADPH, which is necessary for the synthesis of fatty acids and steroids.

The relationships between the pentose-shunt, glycolysis and lipogenesis in isolated fat cells have been shown by Kather et al., (1972). Plots of both pentose phosphate-cycle activity and fatty acid synthesis versus glucose uptake revealed that the extent of glucose uptake, over a wide range, determines the rates of fatty acid synthesis and glucose metabolism via the pentose phosphate-cycle the rate of which is determined by the requirement of NADPH for lipogenesis (Kather et al., 1972). Thus the liver of hedgehog may have a greater lipogenic capacity than that of guinea pig and this effect may be attenuated during the hibernation. As discussed in paper I, Forsberg and Sarajas (1955) found a higher total liver lipid content and an increased incorporation of glucose into the liver lipids in hibernating hedgehogs. Whitten and Klain (1969) observed low values of NADP malic dehydrogenase, citrate cleavage enzyme and G-6-PDH during hibernation of the ground squirrel (Citellus tridecemlineatus). This demonstrates a decreased lipogenic capacity in this species. The great species and organ differences in lipogenic capacities are discussed in paper I.

As discussed in paper I the heart of the hibernator may increase the turnover of lipids during hibernation. The content of lipid droplets increased considerably in the hibernating Citellus lateralis heart in comparison with the non-hibernating state (Burlington et al., 1972), which could not be found in hibernating hedgehog hearts (paper II). If the assumption is correct that the lipogenesis in heart is higher during hibernation and no fat droplets are seen to be accumulated in the heart, then the lipolysis must also be increased during hibernation. This high capacity for lipid turnover in hedgehog heart is supported by the high activity of alpha-GPDH in hedgehog heart in comparison with guinea pig heart during all seasons except autumn (paper I). It has been known for a long time that the heart has a great capacity for oxidizing lipids (Paleus 1966). The pentose-shunt may also be useful for the anaerobic heart as a by-pass to avoid the ATP-consuming phosphorylation of fructose-6-phospate.

4. Enzymatic changes in white and brown adipose tissue.

White and brown adipose tissue of hedgehogs are characterized by a high activity of G-6-PDH at autumn, which is correlated with the high lipogenesis during that season. Besides brown fat G-6-PDH increases at spring, which is also correlated to the higher content of lipids in the brown fat of summer hedgehogs (paper II). The activity of alpha-GPDH of both white and brown adipose tissue have maximal values during spring, which may be an expression of the high lipolytic activity during spring.

The changes of brown fat MDH activity mirror the changes of soluble protein concentration (paper I) and the number of mitochondria (paper II). The brown adipose tissue is an aerobic organ (Lindberg 1970), which also is supported by the low LDH/MDH ratio (paper I) and the relative high LDH-H content (paper III). White adipose tissue of hedgehog, however, had a lower content of LDH-H (paper III) and a higher LDH/MDH ratio (paper I),which speaks in favor of an anaerobic metabolism in white adipose tissue.

5. The change of activation energy (E_a) of LDH and the temperature adaptation of enzymes. (Paper I & III)

As described in paper I there are seasonal changes in the activation energy of LDH from hedgehog heart. The variations are small and have probably no biological significance for the heart of the hibernator (paper I and III), but it is interesting that the variations in activation energy of LDH mirror the changes in the isoenzyme distribution (paper III). In paper III, LDH-H has been shown to have higher activation energy and heat stability than LDH-M, which is supported by many authors (paper III). In consequence, LDH with different isoenzyme distributions from various organs has different activation energies and heat stabilities. This explains the values obtained by Johansson (1969) and also the values, calculated from Burlington and Sampson (1968), which also show that there is a positive correlation between Q_{10} of LDH and the percentage of LDH-H. The lack of difference in the activation energies of LDH and MDH between homeotherms, hibernators and poikilotherms (paper III) show that there is no temperature adaptation in E_a of these enzymes as was shown by Vroman and Brown (1963) and Woodard and Zimny (1973) for succinic dehydrogenase. There are, however, other possibilities for enzymatic temperature adaptation, which are reviewed by Somero (1972) (a) the concentration of enzymes could be varied in a compensatory manner. (b) the enzymes having compensatory differences in catalytic efficiency could be produced at different temperatures. (c) the composition of the catalytic environment could be altered.

The first alternative (a) mentioned by Somero (1972) has never been examined at temperature compensation (Somero 1972). The second alternative (b) has been discussed in paper III, where the value of changing E_a and K_m of enzymes are estimated. The third alternative (c) consists of changes in pH, cations and anions. In this study the temperature dependence of pH optimum of MDH from hibernating hedgehog has been studied and a clear decrease of the pH optimum has been obtained at increasing temperatures. This points to the fact that pH can be a controlling or compensatory factor at cold adaptation as has been shown for citrate synthase of the rainbow trout (Hochachka and Lewis 1970). pH and the concentration of different ions in intracellular compartments are very difficult to measure and there are no information about these things in connection with temperature adaptation.

MDH has been shown to have different temperature activity curves from mammals and poikilotherms (paper III). This is an interesting parallel to acetylcholinesterase (Johansson 1969). The biological significance of these results is probably that more heat resistant enzymes have been evolved in the mammals to resist the higher body temperatures. In contrast to LDH the isoenzymes of MDH from hedgehog and bat (Nyctalus) do not seem to have different E_a (paper III), but the electrophoretic mobility of C-MDH from guinea pig, hedgehog and bat (paper III) suggest that there are differences in E_a of C-MDH Paulsrud et al., (1970) observed a negative correlation between E_a of C-MDH and the anodal electrophoretic mobility of C-MDH from bats and rats and they emphasized that equal energies of activation of C-MDH and M-MDH are important for cold resistance. In addition to this, Kitto and Wilson (1966) found a lower C-MDH mobility of birds capable of cold lethargy. Thus, more experimental evidence from additional species are needed before the theory of Paulsrud et al., (1970) can be accepted.

6. Changes in the ultrastructure of brown adipose tissue and myocardium (Paper II).

The greater number of mitochondria in brown fat during hibernation and spring correlates well with the high protein concentration, the high MDH-activity, the low LDH/MDH ratio, the high percentage of LDH-H and the well-known fact that the aerobic capacity of brown fat is very great (Lindberg 1970). These results are quite in accordance with the thermogenic needs during the arousals from hibernation (Kristoffersson and Soivio 1964, Ball 1965, Smith and Hock 1963). The fat content of brown fat seems to be maximal during summer (Soumalainen and Herlevi 1951, Paleus and Johansson 1968). The lipid from summer animals is concentrated in large fat droplets, which occupy most of the cell volume. During winter and spring the fat content is lower (Paleus and Johansson 1968) and the

lipid is found in many small fat droplets, which may provide easy access for fat catabolism. As discussed in paper II no obvious ultrastructural changes were found in the myocardium of hedgehog or guinea pig. Rosenquist (1970) found that cell coat thickness of myocardium increases in the ground squirrel (Citellus tridecemlineatus) in hibernation. This has been confirmed by Burlington et al., (1972). Burlington et al., (1972) also found 217 % more lipid droplets in the myocardium of the hibernating ground squirrel (Citellus lateralis) compared with non-hibernating ground squirrels. This cannot be supported by the results from hedgehogs, as very few lipid droplets have been seen in the myocardium.

Conclusions.

1. A clear relationship between the activation and inactivation energies of LDH and the isoenzyme distribution of LDH has been established.

2. No differences in activation energy of LDH or MDH have been found from the mammals: guinea pig, hedgehog (hibernating and non-hibernating), bat (*Nyctalus*) (hibernating) or from the poikilotherms: frog and cod.

3. The seasonal changes of LDH, LDH isoenzymes, MDH, MDH isoenzymes, G-6-PDH and alpha-GPDH activities suggest the following seasonal changes in metabolic capacities:
   a. A shift towards more anaerobic metabolism during hibernation.
   b. An increased gluconeogenesis during hibernation.
   c. A high dependence in the liver and perhaps also in the heart of hibernators on the pentose-shunt, especially during hibernation.
   d. High lipogenic capacities in white anc brown fat at autumn and in brown fat also at spring.
   e. High turnover of glycerol (lipids) in white and brown fat during spring.
   f. High aerobic metabolism of brown fat, especially during hibernation and spring.

4. The seasonal changes in the ultrastructure of brown adipose tissue, with a maximal amount of mitochondria in winter and spring and a maximal amount of fat during summer are well correlated to the aerobic metabolism (3f) and the lipogenic capacity (3d).

5. No changes in the ultrastructure of myocardium have been observed.

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Errata.

Paper I:

page 72 line 10: During the winter LDH activity of hedgehog white fat is greater than that in guinea pig.
read: ..... is smaller than that .....

page 76 line 4 from bottom: LDH from the pectoralis muscle of Nyctalus noctula has an even higher value for the heat of activation than LDH from the heart of N. noctula.
read: ..... has about the same value ..... as LDH from .....

page 79 line 7 from bottom: During autumn (I), winter (II) and spring (III) the activity of hedgehog MDH is higher than that of guinea pig.
read: ..... MDH is lower than .....

page 85 line 14: The activity from hedgehog heart is twice as high as that from guinea pig.
read: ..... is half as .....

page 87 line 18: Whitten and Klain (1968 b)
read: Whitten and Klain (1969)

Paper II:

page 127 line 9: Suomalainen and Herlevi (1950)
read: Suomalainen and Herlevi (1951)

In the reference list of paper I & II:

Hiestrand, W.A., W.T. Rockhold, F.W. Stemler, D.E. Stullken and J.E. Wiebers,
read: Hiestand, W.A., et al.,

Moreland J.E. Electronmicroscope studies of mitochondria in cardiac and skeletal muscle from hibernated ground squirrels.
read: Electronmicroscopic .....

Suomalainen P. & A.-M. Herlevi (1950)
read: Suomalainen P. & A.-M. Herlevi (1951).

Whitten B.K. & G.J. Klain (1968 b)
read: Whitten B.K. & G.J. Klain (1969)

Paper III:

page 3 line 11: for the homeotherm and rabbit,
read: for the homeotherm, rabbit,

page 4 line 5 from bottom: rats. Kanugo and .....
read: rats (Burlington and Wiebers 1967). Kanugo and .....

page 6 line 7: All animals were captuered in the wild,
read: ..... were captured in the wild,

page 13 line 6: (Alkinson 1966).
read: (Atkinson 1966).

References.

Aloia R.C. & Pengelley E.T. (1971) Ultrastructure of the ventricular tissue of the hibernating ground squirrel, Citellus lateralis, in relation to the physiology of the hibernators heart. Comp. Biochem, Physiol. 38, 517-524.

Altman M. & Robin E.D. (1969) Survival during prolonged anaerobiosis as a function of an unusual adaptation involving lactate dehydrogenase subunits. Comp. Biochem. Physiol. 30, 1179-1187.

Andjus R.K., Cirković T., Cuperlović N., Davidović J., Marković - Uskoković V. & Velimirović T., (1964) Brain metabolism and resistance of a hibernator (Citellus citellus) and the rat to different anoxic conditions, including cardiac arrest in deep hypothermia. Ann. Acad. Sci. Fenn., Ser. A., IV. 71, 11-23.

Arinze I.J., Garber A.J. & Hanson R.W. (1973) The regulation of gluconeogenesis in mammalian liver. The role of mitochondrial phosphoenolpyruvate carboxykinase. J. biol. Chem. 248, 2266-2274.

Ball E.G. (1965) Some energy relationships in adipose tissue. Ann. N.Y. Acad. Sci. 131, 225-234.

Ballard F.J., Hanson R. W. (1967) Phosphoenolpyruvate carboxykinase and pyruvate carboxylase in developing rat liver. Biochem. J. 104, 866-871.

Barker W. (1958) Winter sleep. In The Illustrated Library of the Natural Sciences. (Edited by Weyer E. M.) II, 1284-1293. Simone & Schuster, New York.

Barkow H.C.L. (1846) Der Winterschlaf. 525 pages. Aug. Hirschwald, Berlin.

De Beauvais Vincent (1470-71) Speculum Majus I, Speculum naturale. Chap. 131. Incunabulum 1470. Bibliothèque Nat. Univ. Strasbourg. cit. from Kayser (1961) page 194.

Benedict F.G. & Lee R.C. (1938) Hibernation and marmot physiology. Carnegie Inst. Washington, Washington DC Publ. no 497. 239 pages. Waverly Press Inc. Baltmore.

Björck G., Johansson B.W. & Schmid H. (1956) Reactions of hedgehogs, hibernating and non-hibernating, to the inhalation of oxygen, carbon dioxide and nitrogen. Acta Physiol. scand. 37, 71-83.

Blatt W.F., Walker J. & Mager M. (1965) Tissue lactic dehydrogenase isozymes: Variation in rats during prolonged cold exposure. Amer. J. Physiol. 209, 785-789.

Blix A.S. & From S.Hj. (1971) Lactate dehydrogenase in diving animals - a comparative study with special reference to the eider (Somateria mollissima). Comp. Biochem. Physiol. 40 B, 579-584.

Brush A.H. (1968) Response of isozymes to torpor in the bat, Eptesicus fuscus. Comp. Biochem. Physiol. 27, 113-120.

Burlington R.F. (1972) Recent advances of intermediary metabolism of hibernating mammals. In Hibernation-Hypothermia, perspectives and challenges. (Edited by South F.E., Hannon J.P., Willis J.R., Pengelley E.T. & Alpert N.R.) 3-15, Elsevier, Amsterdam.

Burlington R.F., Bowers W.D., Daum R.C. & Ashbaugh P. (1972) Ultrastructural changes in heart tissue during hibernation. Cryobiology 9, 224-228.

Burlington R.F. & Klain G.J. (1967) Gluconeogenesis during hibernation and arousal from hibernation. Comp. Biochem. Physiol. 22, 701-708.

Burlington R.F. & Sampson J.H. (1968) Distribution and activity of lactic dehydrogenase isozymes in tissues from a hibernator and non-hibernator. Comp Biochem. Physiol. 25, 185-192.

Burlington R. F. & Wiebers J.E. (1966) Anaerobic glycolysis in cardiac tissue from a hibernator and non-hibernator as effected by temperature and hypoxia. Comp. Biochem. Physiol. 17, 183-189.

Burlington R. F. & Wiebers J. E. (1967) The effect of temperature on glycolysis in brain and skeletal muscle from a hibernator and a non-hibernator. Physiol. Zool. 40, 201-206.

Bücher T. & Klingenberg M. (1958) Wege des Wasserstoffs in der lebendigen Organization. Angew. Chem. 70, 552-570.

Cade T.J. (1964) The evolution of torpidity in rodents. In Mammalian Hibernation II (Edited by Suomalainen P.) Ann. Acad. Sci. Fenn. Ser A IV 71, 77-112.

Cahn R.D. (1963) Cellular damage and the control of lactic dehydrogenase synthesis in cell cultures by oxidative metabolites. J. Cell. Biol. 19, (2) 12 A.

Cahn R.D. (1964) Developmental changes in embryonic enzyme patterns: the effect of oxidative substrates on lactic dehydrogenase in beating chick embryonic heart cell cultures. Devel. Biol. 9, 327-346.

Cahn R.D., Kaplan N.O., Levine L. & Zwilling E. (1962) Nature and development of lactic dehydrogenases. Science 136, 962-969.

Chappell J.B. (1968) Systems used for the transport of substrates into mitochondria. Brit. Med. Bull. 24, 150-157.

Clark W.M. (1928) The determination of hydrogen ions. sid 206. Baillière, Tindall and Cox London.

Covino B.G. & Hannon J.P. (1959) Myocardial metabolic and electrical properties of rabbits and ground squirrels at low temperatures. Amer.J.Physiol. 197, 494-498.

Dawson D.M., Goodfriend T.L. & Kaplan N.O. (1964) Lactic dehydrogenases: functions of the two types rates of synthesis of the two major forms can be correlated with metabolic differentiation. Science (N.Y.) 143, 929-933.

Denyes A. & Carter J.D. (1961) Utilization of acetate-1-C^{14} by hepatic tissue from cold-exposed and hibernating hamsters. Amer. J. Physiol. 200, 1043-1046.

Dorst J. (1962) Les migrations des oiseaux. 430 pages. Payot 25. Paris.

Eisentraut M. (1956) Der Winterschlaf mit seinen ökologischen und physiologischen Begleiterscheinungen. 160 pages. VEB,Gustav Fischer Verlag, Jena.

Everse J. & Kaplan N.O. (1973) Lactate dehydrogenases: structure and function. Adv. Enzym. 37, 61-133.

Fahien L.A. & Strmecki M. (1969) Studies of gluconeogenic mitochondrial enzymes. IV The conversion of oxaloacetate to fumarate by bovine liver mitochondrial malate dehydrogenase and fumarase. Arch. Biochem. Biophys. 130, 478-487.

Forsberg A. & Sarajas H.S.S. (1955) Studies on the metabolism of ^{14}C-labelled glucose in awake and hibernating hedgehogs. Ann. Acad. Sci. Fenn. Ser. A IV 28, 3-8.

Galster W. A. & Morrison P. (1970) Cyclic changes in carbohydrate concentrations during hibernation in the arctic ground squirrel. Amer J. Physiol. 218, 1228-1232.

Garber A.J., Ballard F.J. & Hanson R.W. (1972) In Proceedings of symposium on metabolic regulation, energy metabolism and the regulation of metabolic processes in mitochondria (Edited by Mehlman M.A. & Hanson R.W.) p. 109. Academic Press New York.

Goodfriend T.L. & Kaplan N.O. (1963) Induction of changes in the subunit composition of Lactic dehydrogenase . J. Cell. Biol. 19, (2) 28 A.

Gordon M.S., Bartholomew G.A., Grinnell A.D., Jörgensen C.B. and White F.N. (1972) Animal physiology: principles and adaptations. Chapter 8, 298-368. The Macmillan Company New York.

Griffin D.R. (1970) Migrations and homing of bats. In Biology of bats. (Edited by Wimsatt W.) Vol I. Ch.7., 233-264. Academic Press, New York.

Hannon J.P. (1958) Effect of temperature on the heart rate, electrocardiogram and certain myocardial oxidations of the rat. Circulat. Res. 6, 771-778.

Hannon J.P., Beyer R.E., Burlington R.F., Somero G.N. & Bland J.H. (1972) A guide for future studies of low temperature metabolic function. In Hibernation - Hypothermia, perspectives and challenges. (Edited by South F.E., Hannon J.P., Willis J.R., Pengelley E.T. & Alpert N.R.) 99-120. Elsevier Amsterdam.

Hannon J.P., Vaughan D. A. & Hock R. (1961) Endogenous tissue respiration of the arctic ground squirrel as affected by hibernation and season. J. cell. comp. Physiol., 57, 5.

Hanson R.W. & Garber A.J. (1972) Phosphoenolpyruvate carboxykinase. I Its role in gluconeogenesis. Amer. J. clin.Nutr. 25, 1010-1021.

Hayward J.S, & Lyman C.P. (1967) Nonshivering heat production during arousal from hibernation and evidence for the contribution of brown fat. In Mammalian Hibernation III. (Edited by Fisher K.C., Dawe A.R., Lyman C.P., Schönbaum E. & South F.E.) 346-355. Oliver & Boyd, Edinburgh.

Helle W. (1953) L'influence du taux élevé de glucose sanguin sur le sommeil hivernal des hamsters (Mesocricetus auratus). Physiologia comp. Oecol. 3, 190-196.

Hensel H., Brück K. & Raths P. (1973) Homeothermic organisms. In Temperature and Life (Edited by Precht H., Christophersen J., Hensel H. & Larcher W.) 503-761. Springer Verlag Berlin.

Hiestand, W.A., Rockhold W.T., Stemler F.W., Stullken D.E. & Wiebers J.E. (1950) The comparative hypoxic resistance of hibernators and non-hibernators. Physiol. Zool. 23, 264-268.

Hochachka P.W. & Lewis J.K. (1970) Enzyme variants in thermal acclimation: trout liver citrate synthases. J. biol. Chem. 245, 6567-6573.

Huttonen M. & Johansson B.W. (1963) The influence of the dietary fat on the lethal temperature in the hypothermic rat. Acta Physiol. scand. 59, 7-11.

Irving L. (1964) Terrestrial animals in cold: birds and mammals. In Handbook of Physiology (Edited by Dill D.B., Adolph E.F. & Wilber C.G.) Section 4: Adaptation to the Environment. 361-377. Amer. Physiol. Soc. Washington D.C.

Jankowsky H.-D. (1973) Body temperature and external temperature. In Temperature and life. (Edited by Precht H., Christophersen J., Hensel H. & Larcher W.) 293-301. Springer Verlig Berlin.

Johansson B.W. (1967) Heart and circulation in hibernators. In Mammalian Hibernation III (Edited by Fischer K.C., Dawe A.R., Lyman C.P., Schönbaum E. & South F.E.) 200-218, Oliver & Boyd, Edinburgh.

Johansson B.W. (1969) Temperature dependence of cholinesterase and lactate dehydrogenase in the guinea-pig, hedgehog and codfish. Acta physiol. scand. 77, 1-6.

Kalabuchov N.I. (1956) Hibernation of animals (Russ) 267 pages Gorki State Univ. Press. Charkov.

Kaplan N.O. (1964) Lactate dehydrogenase - structure and function. Brookhaven Symp. Biol. 17, 131-153.

Kather H., Rivera M. & Brand K. (1972 a) Interrelationship and control of glucose metabolism and lipogenesis in isolated fat - cells. Effect of the amount of glucose uptake on the rates of the pentose phosphate cycle and of fatty acid synthesis. Biochem. J. 128, 1089-1096.

Kather H., Rivera M. & Brand K. (1972 b) Interrelationship and control of glucose metabolism and lipogenesis in isolated fat - cells. Control of pentose phosphate-cycle activity by cellular requirement for reduced nicotinamide adenine dinucleotide phosphate. Biochem. J. 128, 1097-1102.

Kayser C. (1961) The physiology of natural hibernation. 325 pages, Pergamon Press, Oxford.

Kayser C. & Malan A. (1963) Central nervous system in hibernation. Experientia 19, 441-451.

Kemp F.D. (1969) Thermal reinforcement and thermoregulatory behaviour in the lizard Dipsosaurus dorsalis: an operant technique. Anim. Behav. 17, 446-451.

Kitto B.B. & Wilson A.C. (1966) Evolution of malate dehydrogenase in birds. Science (N.Y.) 153, 1408-1410.

Klain G.J. & Rogers G.B. (1970) Seasonal changes in adipose tissue lipogenesis in the hibernator. Intern. J. Biochem. 1, 248-250.

Klain G.J. & Whitten B.K. (1968) Carbon dioxide fixation during hibernation and arousal from hibernation. Comp. Biochem. Physiol. 25, 363-366.

Kleiber M. (1947) Body size and metabolic rate. Physiol. Rev. 27, 511-541.

Kristoffersson R. & Soivio A. (1964) Hibernation of the hedgehog (Erinaceus europaeus L.). The periodicity of hibernation of undisturbed animals during the winter in a constant ambient temperature. Ann. Acad. Sci. Fenn. Ser. A. IV, 80, 1-22.

Lindberg O. ed. (1970) Brown adipose tissue. 337 pages, Elsevier, New York.

Lindy S. & Rajasalmi M. (1966) Lactate dehydrogenase isozymes of chick embryo: response to variations of ambient oxygen tension. Science (N.Y.) 153, 1401-1402.

Linnaeus Carolus (1758) Systema naturae per regna tria naturae, secundum classes, ordines, genera, species cum characteribus, differentiis, synonymis, locis. Editio decima, reformata, Stockholm, Laurentii Salvii, vol. 1, (ii) + 824 pp.

Lybecker 85 (c.1715) cit. from Ordbok öfver Svenska Språket utgifven af Svenska Akademien. 7, D 2403. Gleerups förlag, Lund 1925.

Lyman C.P. (1970) Thermoregulation and metabolism in bats. In Biology of bats. (Edited by Wimsatt W.A.) 1, 301-330. Academic Press, New York.

Lyman C.P. & Chatfield P.O. (1950) Mechanisms of arousal in the hibernating hamster. J. exp. Zool. 114, 491-516.

Lyman C.P. & Leduc E.H. (1953) Changes in blood sugar and tissue glycogen in the hamster during arousal from hibernation. J. cell. comp. Physiol. 41, 471-491.

Manasek F.J., Adelstein S.J. & Lyman C.P. (1965) The effects of hibernation on the in vitro synthesis of DNA by hamster lymphoid tissue. J. cell. comp. Physiol. 65, 319-324.

McMurchie E.J., Raison J.K. & Caincross K.D. (1973) Temperature - induced phase changes in membranes of heart: a contrast between the thermal response of poikilotherms and homeotherms. Comp. Biochem. Physiol. 44B, 1017-1026.

Moreland J.E. (1962) Electronmicroscopic studies of mitochondria in cardiac and skeletal muscle from hibernated ground squirrels. Anat. Rec. 142, 155-160.

Nielsen K.C. (1968) Experimental studies on the involvement of adrenergic mechanisms in the development of ventricular fibrillation during induced hypothermia. Studentlitteratur, Lund 1968, 1-39.

Nordlie R.C. & Landy H.A. (1963) Mammalian liver phosphoenolpyruvate carboxykinase activities. J. biol. Chem. 238, 2259-2263.

Norris K.S. (1953) The ecology of the desert iguana (Dipsosaurus dorsalis). Ecology 34, 265-287.

Paleus S. (1966) Myokardiets metabolism. Nordisk Medicin 75, 4-10.

Paleus S. & Johansson B.W. (1968) Seasonal variations in cytochrome c content of hedgehog brown adipose tissue. Acta chem. scand. 22, 342-344.

Paulsrud J.R., Mann K.G. & Dryer R.L. (1970) A comparison of rat and bat malic dehydrogenase isoenzymes. In Brown Adipose Tissue. (Edited by Lindberg O.) Chap. 8. 197-206. Elsevier New York.

Pfleiderer G. & Wachsmuth E.D. (1961) Alters- und funktionsabhängige Differenzierung der Laktatdehydrogenase menschlicher Organe. Biochem. Z. 334, 185-198.

Poche R. (1959) Elektronenmikroskopische Untersuchungen zur Morphologie des Herzmuskels vom Siebenschläfer während des aktiven und des lethargischen Zustandes. Z. Zellforsch. 50, 332-360.

Rosenquist T.H. (1970) Ultrastructural changes in the plasma membrane and sarcoplasmic reticulum of myocardial cells during hibernation. Cryobiology 7, 14-18.

Rosenquist T.H. & Zimny M.L. (1969) Modification of the cell surface and the muscular triad in the heart of a hibernator. The Physiologist 12, 343.

Salthe S.N. (1965) Comparative catalytic studies of lactic dehydrogenases in the amphibia: environmental and physiological correlations. Comp. Biochem. Physiol. 16, 393-408.

Scrutton M.C. & Utter M.F. (1968) The regulation of glycolysis and gluconeogenesis in animal tissues. Ann. Rev. Biochem. 37, 249-302.

Smalley R.L. & Dryer R.L. (1967) Brown fat in hibernation. In Mammalian Hibernation III (Edited by Fisher K.C., Dawe A.R., Lyman C.P., Schönbaum E. & South F.E.) 325-345, Oliver & Boyd, Edinburgh.

Smit-Vis J.H. & Smit G.J. (1963) Occurence of hibernation in the golden hamster, Mesocricetus auratus Waterhouse. Experientia 19, 363-364.

Smith R.E. & Hock R.J. (1963) Brown fat: thermogenic effector of arousal in hibernators. Science 140, 199-200.

Somero G.N. (1972) Molecular mechanisms of temperature compensation in aquatic poikilotherms. In Hibernation - Hypothermia, perspectives and challenges. (Edited by South F.E., Hannon J.P., Willis J.R., Pengelley E.T., Alpert N.R.) 55-80. Elsevier, Amsterdam.

South F.E. (1958) Rates of oxygen consumption and glycolysis of ventricle and brain slices, obtained from hibernating and non-hibernating mammals, as function of temperature. Physiol. Zool. 31, 6-15.

South F.E. (1960 a) Hibernation, temperature and rates of oxidative phosphorylation by heart mitochondria. Amer. J. Physiol. 198, 463-466.

South F.E. (1960 b) Some metabolic specializations in tissues of hibernating mammals. In Mammalian Hibernation (Edited by Lyman C.P. & Dawe A.R.) Bull. Mus. comp. Zool. (Harv.) 124, 475-492.

South F.E. & House W.A. (1967) Energy metabolism in hibernation. In Mammalian Hibernation III (Edited by Fischer K.C., Dawe A.R., Lyman C.P., Schönbaum E. & South F.E.) 305-324. Oliver & Boyd, Edinburgh.

Studier E.H. (1974) Differential in rectal and chest muscle temperature during arousal in Eptesicus fuscus and Myotis sodalis (Chiroptera: Vespertilionidae) Comp. Biochem. Physiol. 47 A, 799-802.

Suomalainen P. & Herlevi A.-M. (1951) The hibernating gland and the alarm reaction. Arch. Soc. Zool. Botan. Fenn. "Vanamo" 5, (2) 72-73.

Tait J. (1922) The heart of hibernating animals. Amer. J. Physiol. 59, 467.

Tashima L.S., Adelstein S.J. & Lyman C.P. (1970) Radioglucose utilization by active, hibernating, and arousing ground squirrels. Amer. J. Physiol. 218, 303-309.

Thorell J., Johansson B.W. & Senturia J.B. (1972) Carbohydrate metabolism. In. Seasonal variations in the physiology and biochemistry of the european hedgehog (Erinaceus europaeus) including comparisons with non-hibernators, guinea-pig and man. (Edited by Johansson B.W. & Senturia J.B.) Acta Physiol. scand. Suppl. 380, 43-48.

Tolstoj L.N. cit. from Kalabuchov (1956) Hibernation of animals (Russ). p. 89, Gorki State Univ. Press, Charkov.

Vesell E.S. & Pool P.E. (1966) Lactate and pyruvate concentrations in exercised ischemic canine muscle: relationship of tissue substrate level to lactate dehydrogenase isozym pattern. Proc. nat. Acad. Sci. 55, 756-762.

Whitten B.K. & Klain G.J. (1968) Protein metabolism in hepatic tissue of hibernating and arousing ground squirrels. Amer. J. Physiol. 214, 1360-1362.

Whitten B.K. &Klain G.J. (1969) NADP specific dehydrogenases and hepatic lipogenesis in the hibernator. Comp. Biochem. Physiol. 29, 1099-1104.

Wilson A.C., Cahn R.D. & Kaplan N.O. (1963) Functions of the two forms of lactic dehydrogenase in the breast muscle of birds. Nature (Lond.) 197, 331-334.

Wilson A.C., Kaplan N.O., Levine L., Pesce A., Reichlin M. & Allison W.S. (1964) Evolution of lactic dehydrogenases. Fed. Proc. 23, 1258-1266.

Woodard C.B. & Zimny M.L. (1973) Energy of activation of succinic dehydrogenase in a hibernator. Amer. J. Physiol. 208, 1247-1252.

Vroman C.B. & Brown J.R.C. (1963) Effect of temperature on the activity of succinic dehydrogenase from the livers of rats and frogs. J. cell. comp. Physiol. 6, 129-131.

Wuntch T., Vesell E.S. & Chen R.F. (1969) Studies of rates of abortive ternary complex formation of lactate dehydrogenase isozymes. J. biol. Chem. 244, 6100-6104.

Wuntch T., Chen R.F. & Vesell E.S. (1970 a) Lactate dehydrogenase isozymes: kinetic properties at high enzyme concentrations. Science 167, 63-65.

Wuntch T., Chen R.F. & Vesell E.S. (1970 b) Lactate dehydrogenase isozymes: further kinetic studies at high enzyme concentrations. Science 169, 480-481.

Zimny M.L. & Moreland J.E. (1968) Mitochondrial populations and succinic dehydrogenase in the heart of a hibernator. Can. J. Physiol. Pharmacol. 46, 911-913.

Ulfstrand S. (1963) Fåglar 3. In Djurens värld (Edited by Hanström B.) 10, 119-125. Förlagshuset Norden AB, Malmö.

КАК ОНО СХВАТИЛА
МЕНЯ.., СХВАТИЛА,
СХВАТИЛА, ПОВАЛИЛА
МЕНЯ НАЗЕМЬ,
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Seasonal variations in the physiology and biochemistry of the european hedgehog (Erinaceus europaeus) including comparisons with non-hibernators, guinea-pig and man.

Edited by

BENGT W. JOHANSSON

and

JEROME B. SENTURIA

MALMÖ 1972

Chapter 11

Dehydrogenases (LDH, MDH, G-6-PDH and α-GPDH) in the Heart, Liver, White and Brown Fat

by

Sven-Olle R. Olsson

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I. INTRODUCTION

The dehydrogenases studied here have more or less key positions in the carbohydrate metabolism as is illustrated in figure 11.1.

Lactic dehydrogenase (LDH) (EC 1.1.27) catalyzes the final step in anaerobic catabolism of glucose.

Malic dehydrogenase (MDH) (EC 1.1.1.37) participates in the Krebs'cycle, which is of importance for aerobic catabolism. MDH also functions in gluconeogenesis.

Glucose-6-phosphate dehydrogenase (G-6-PDH) (EC 1.1.1.49) catalyzes the first step in the pentose-shunt, which is important for the production of $NADPH_2$ and ribose.

α-Glycerophosphate dehydrogenase (α-GPDH) (EC 1.1.1.8) links glycerol catabolism with glycolysis.

These four enzymes were studied in hedgehogs both hibernating and non-hibernating and in guinea-pigs, which were killed during four periods of the year (Chapter 2).

The multiple molecular forms (isoenzymes) of LDH and MDH gained particular interest.

One of the most obvious differences between hibernators and non-hibernators is the cold-resistance of tissues from hibernators. These tissues function at much lower temperatures than do those of non-hibernators. The organ which has been studied most extensively in this respect is heart (Johansson 1967, 1969a). Mokrash et al. (1960) reported lower Q_{10}-values for heart rate in isolated preparations from hibernating hamsters than from non-hibernating hamsters and rats but these results are not supported by Michael and Menaker (1963) or Senturia et al. (1970), who worked with bats and ground squirrels respectively. The Q_{10}-values for oxygen consumption of ventricles have also been found to vary. The value for rat ventricles was found to be higher than that for non-hibernating hamster ventricles, which in turn is higher than the values from hibernating hamsters and bats (South 1958). Essentially the same results have been found from rats and hibernating and non-hibernating hamsters for oxygen consumption of isolated mitochondria from ventricles (South 1960 a and b) and for esterification of inorganic phosphate by mitochondria of the ventricles (South 1960 a and b). These results show that there is an enzymatic difference in temperature resistance between the non-hibernator (rat) and the hibernator (hamster), and that in hamsters there evidently is an enzymatic adaptation to hibernation.

During recent years many enzymes have been found to be composed of different protein structures (isoenzymes), which, however, catalyze the same reaction (Wilkinson 1965 and Latner and Skillen 1968). The enzyme which is best known in this respect is Lactic dehydrogenase. LDH is composed of five isoenzymes and every isoenzyme consists of a tetramer. Two types of subunits H and M are randomly distributed in the five tetramers (isoenzymes). LDH-1 consists of four H-subunits and LDH-5 consists of four M-subunits. The other three isoenzymes consist of 3H and 1M (LDH-2), 2H and 2M (LDH-3) and 1H and 3M (LDH-4). These five isoenzymes are distributed according to the binomial distribution in each kind of cell (Markert 1963, Kaplan and Cahn 1962

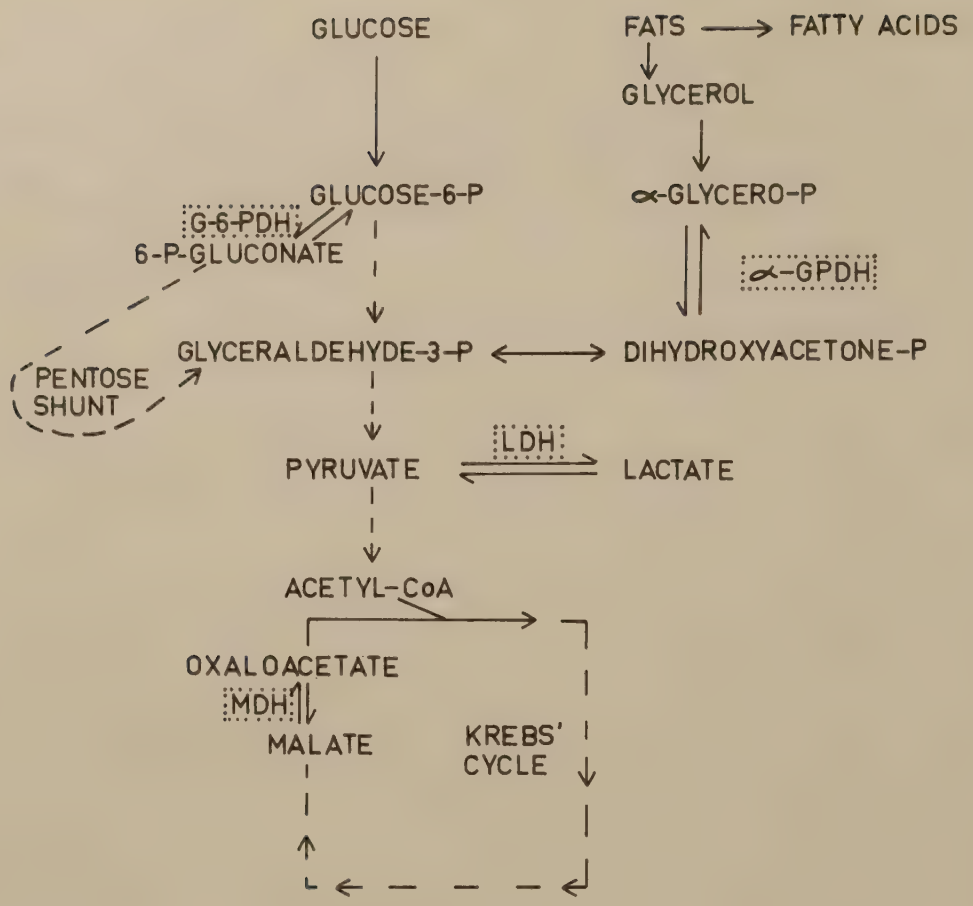

GLUCOSE
FATS
FATTY ACIDS
GLYCEROL
GLUCOSE-6-P
G-6-PDH
6-P-GLUCONATE
α-GLYCERO-P
α-GPDH
PENTOSE SHUNT
GLYCERALDEHYDE-3-P
DIHYDROXYACETONE-P
LDH
PYRUVATE
LACTATE
ACETYL-CoA
OXALOACETATE
MDH
MALATE
KREBS' CYCLE

Fig. 11.1

and Boyer et al. 1963).

If the relative activities of the two subunits are H and M (H + M = 1), the activities of the isoenzymes are the expansion of $(H + M)^4$. Palmer and Karlsson (1969) have shown that liver cells free from connective tissue have an LDH-distribution, which fits the binomial distribution better than LDH from whole liver or from connective tissue. Their conclusion is that each cell line has a specific ratio of H/M and a binomial distribution of the activities of H and M. The synthesis of two subunits is controlled by different genes, whose changes in activity result in changes in the relative activities of the isoenzymes. These changes have been demonstrated during development of mammals (Karlsson and Palmer 1971).

The distribution of LDH-isoenzymes in different organs has been correlated to the metabolic functions of that organ. Dawson et al. (1964) pointed out that organs with anaerobic metabolism have a high proportion of LDH-M (e.g. skeletal muscle and white fat) and that organs with aerobic metabolism have a hig proportion of LDH-H (e.g. heart and brain). Wilson et al. (1963) investigated the LDH-isoenzymes from the pectoralis muscle from more than 30 species of birds and found that skillful flyers such as swifts had a higher proportion of LDH-H than domestic fowl and game birds. It is generally accepted that muscles which work more or less continuously (i.e. the myocardium or pectoralis muscle) have a higher activity of LDH-H than those muscles which work intermittently such as extremity muscles (Dawson et al. 1964, Kaplan 1964, Lindsay 1963). The later group of muscles with high proportion of LDH-M will produce lactate and increase the oxygen debt at increased work. The reason for this is that LDH-H is inhibited more by increasing concentrations of pyruvate than LDH-M (Cahn et al. 1962). Salthe (1965) found that there is a difference between the distribution of LDH in terrestrial aerobic-living and in aquatic anaerobic living amphibians. In vitro, the synthesis of LDH-M is inhibited (Goodfriend and Kaplan 1963, Dawson et al. 1964) and synthesis of LDH-H activated by increasing the PO_2 (Cahn 1963, 1964). Lindy and Rajasalmi (1966) incubated eggs in hypoxic and hyperoxic environments. The hypoxic eggs had a higher activity of LDH-M than the hyperoxic eggs. It is possible that oxygen content in the tissues may be a regulating factor for the synthesis of LDH-H and LDH-M and that tissues with aerobic metabolism have a high activity of LDH-H. Burlington and Wiebers (1966) showed that the capacity of the myocardium for anaerobic glycolysis increased during hibernation and that this increased capacity is in connection with the hypoxic tolerance of the hibernators (Björck et al. 1956a, Kayser and Malan 1963, Andjus et al. 1964). Burlington and Sampson (1968) found that LDH-M increased in the liver, brain and heart of the hibernating ground squirrel (Citellus tridecemlineatus).

Fig. 11.1. A schematic drawing of the principal metabolic pathways in which LDH, MDH, G-6-PDH and α-GPDH are involved.

Malate dehydrogenase consists of two isoenzymes, cytoplasmic (C-MDH) and mitochondrial (M-MDH). Kaplan (1963) proposed the following metabolic role for these isoenzymes: C-MDH reduces oxaloacetate and M-MDH oxidizes malate. A similar role for the mitochondrial and cytoplasmatic α-GPDH has been proposed by Bücher and Klingenberg (1958) and Sacktor (1959).

In this study enzymatic adaptations to hibernation and temperature resistance has been investigated in the following ways:

1. The activities of LDH, MDH, G-6-PDH and α-GPDH from the heart, liver, white and brown fat of the hedgehog were compared to the same organs of the guinea-pig.

2. The seasonal changes of these enzymes in the above mentioned organs were examined.

3. The temperature dependence of the activity of LDH from the heart of the hedgehog was determined.

4. The annual changes and differences of the isoenzymes of LDH and MDH from the heart of the hedgehog and guinea-pig, were also studied.

General methods

The tissue samples from heart, liver, white and brown fat which were taken from hedgehogs and guinea-pigs (Chapter 2), were homogenized with a knife homogenizer (Sorvall) in distilled water to produce 10% (by weight) homogenates. These were centrifuged at 2000 x G for 30 minutes at 4° C and the supernatants were saved for determination of enzyme activities and isoenzyme distribution.

Determination of the concentrations of soluble proteins. Soluble proteins were determined in order to get a rough estimation of their concentration changes with a technically simple and quick method in order to get a point of reference for the enzyme changes (Palmer and Karlsson 1969, Karlsson and Palmer 1971). As this method, described by Layne (1957) is unspecific, only large changes can be observed. The standard deviation in per cent of the concentration of soluble proteins in the different organs is 30% $\pm$ 10%. This is an expression of the individual variations and the inaccuracy of the method. A control experiment with 12 pieces of liver from one guinea-pig gave a standard deviation of 23% , which is less than the value (30%) for all 48 livers of the guinea-pigs. This means that the inaccuracy of the method of determination contributes greatly the standard deviations.

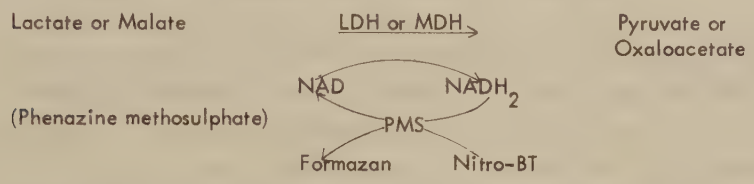
Determination of the enzyme activities. The activities of the dehydrogenases: lactic dehydrogenase (LDH) (EC 1.1.1.27), malic dehydrogenase (MDH) (EC 1.1.1.37), glucose-6-phosphate dehydrogenase (G-6-PDH) (EC 1.1.1.49) and α-glycerophosphate dehydrogenase (α-GPDH) (EC 1.1.1.8) were determined spectrophotometrically (Unicam SP 800-A). The activity was measured from the initial velocity of reduction of the reactions: $NAD \rightarrow NADH_2$ and $NADP \rightarrow NADPH_2$ at 340 mμ using distilled water as reference. The reactions were performed

at 30.0° C ± 0.05° C.

The reactions were started by adding the supernatant of the homogenate to the rest of the reaction mixture. It took about ten seconds to add the supernatant and shake the cuvettes before the automatic spectrophotometric measurement started. If the activity was too high i.e. the change of extinction during the first minute was non-linear, the supernatant was diluted until a linear curve was obtained. The changes for extinction were continuously registered for three to five minutes.

The activities are expressed in activity/wet-weight, the change in extinction during one minute per 10 µl 10% supernatant.

Determination of the isoenzyme distribution, LDH and MDH from the heart. Horizontal starch gel electrophoresis was used (Fine et al. 1963), as modified by Karlsson and Carlsson (1968). The electrophoretic separation were run for 18 hours at 4° C using constant current of 15mA per gel. Four degrees Celsius was selected to avoid heat inactivation of the enzymes. The development of the isoenzyme bands is based on the reactions:



These reactions were stopped after 20 minutes by incubation at 20° C with 3% aqueous formaldehyde.

The amount of insoluble Formazan in the bands was spectrophotometrically measured with a Chromoscan densitometer (Joyce, Loebl and Co. Ltd.), and taken as a measure of the activity of the individual isoenzymes. Latner and Skillen (1968) and Palmer and Karlsson (1969) noted that the absolute activity of the LDH-isoenzyme cannot be considered directly proportional to the amount of dye in all experiments performed. Considering this the activity of the individual isoenzymes are expressed as relative percentage of the total area of the densitogram.

II. PROTEIN

Method

The determination of the soluble protein concentration (mg/10 µl) in the homogenate supernatants was performed by the spectrophotometric method of Layne (1957) using a Beckman DB spectrophotometer.

Results

Heart. See Fig. 11.2.

Liver. See Fig. 11.3.

White fat. See Fig. 11.4.

Brown fat. See Fig. 11.5.

Comparison between the organs. The highest concentration of soluble proteins is found in liver and brown fat from the hedgehog and the liver of the guinea-pig.

Discussion

The changes in the concentration of the soluble proteins from these four organs cannot definitely be correlated with the weight of the organ (Chapter 3) or the concentration of glycogen in the heart and the liver (Chapter 8). Whitten and Klain (1968a) found a decrease in protein synthesis methionine in hepatic tissue of hibernating ground squirrels (Citellus tridecemlineatus) at 37° C compared to the non-hibernatory state, whereas no change was observed at 6° C. Whitten (1971) has also observed that decreased protein synthetic capacity in hibernating animals was primarily due to ribosome disaggregation rather than a change in the activity of cell sap enzymes.

The high concentration of proteins in the brown fat of the hedgehog during hibernation and post hibernation is in agreement with the results of Paleus and Johansson (1968), who found a higher lipidfree dry weight during hibernation in hedgehogs, and Feist and Quay (1969), who found an increased concentration of proteins and a decreased concentration of lipids in cold-acclimated, hibernating and aroused hamsters (Mesocricetus). This is an expression of the higher

---

Fig. 11.2. Concentration of soluble proteins in mg/10 µl. The season refer to the prehibernating (I), hibernating (II), posthibernating (III) and non-hibernating (IV) phase. See further Fig. 2.1. The circles represent the mean and the straigt lines represent the standard deviations solid line hedgehog, interrupted line guinea-pig.

Fig. 11.3. For legends see Fig. 11.2.

Fig. 11.4. For legends see Fig. 11.2.

Fig. 11.5. For legends see Fig. 11.2.

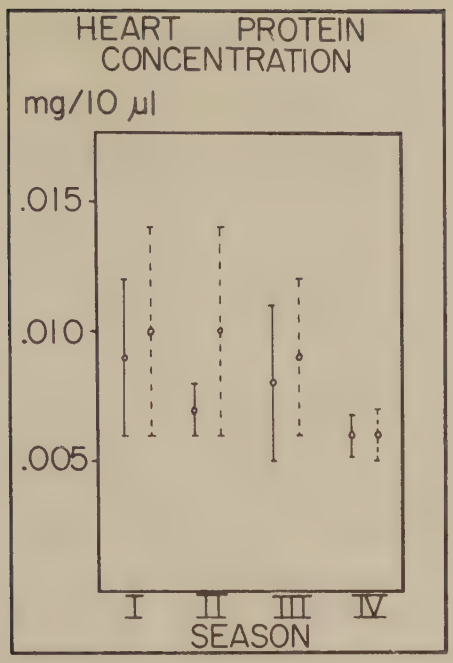
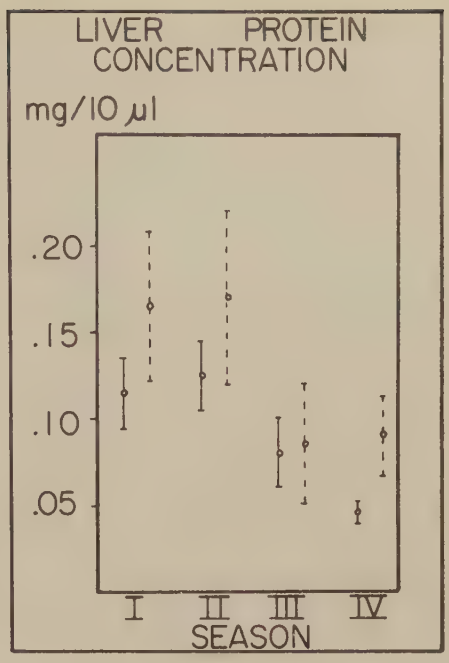


Fig. 11.2



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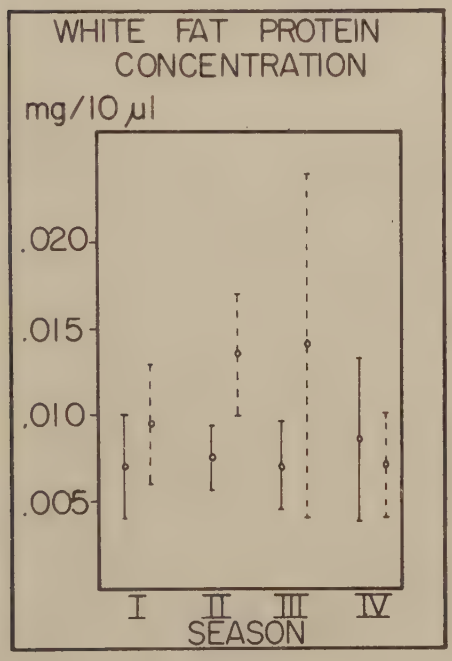

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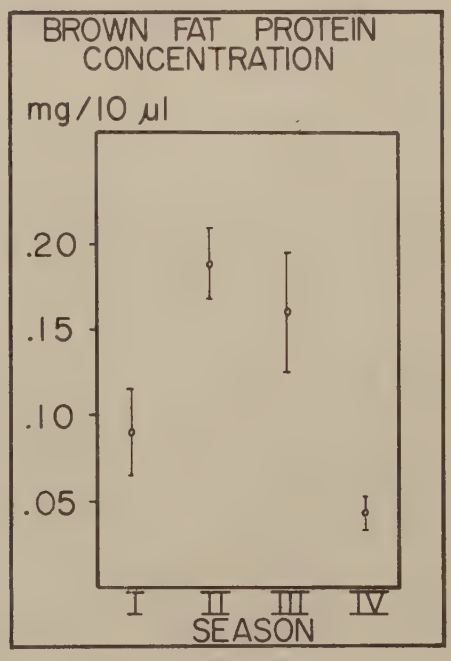

Fig. 11.5

metabolic activity of brown fat during hibernation and post hibernation (Draskoczy and Lyman 1965, Edwards and Munday 1969a and Chapter 15).

It appears to be more relevant to express the activities of enzymes in activity/wet-weight of the organs rather than in activity/mg protein, since the efficiency of the enzymes for the whole organ depend primarily on the total activity of the enzymes of the organ. If there are great changes in the concentration of soluble proteins (which is actually the case in liver, heart and white fat of guinea-pig and in brown fat of hedgehog), the activity/mg protein of enzymes does not reflect the changes in importance of enzymes for the organs, but reflects the changes in concentrations of soluble proteins. Another method of expressing activity of enzymes is activity/dry weight, and according to Kristoffersson et al. (1965) the per cent water content of heart, liver, white and brown fat do not vary more than two per cent of the wet-weight. Paleus and Johansson (1968) found that water content in per cent of wet-weight of brown fat of hedgehogs varies maximally 14% during the year. The conclusions of these results are that variations in water content in these organs do not have a significant influence on the values of activity/wet-weight.

Methods

Determination of the activity of the enzym. The general principles of determination of dehydrogenase activity have already been discussed. The reaction mixture for the spectrophotometric measurement was as follows:

10 µl homogenate supernatant

2.5 ml 0.1M Tris-buffer, pH 8.9

0.3 ml 3M Li-lactate (B.D.H. or Sigma)

0.2 ml NAD 10 mg/ml (Sigma)

The reaction was started by adding the supernatant of the homogenate to the rest of the reaction-mixture.

Determination of the relative activity of the isoenzymes. The technique of electrophoresis was modified after Fine et al. (1963). The starch gel (Connaught Med. Res. Lab., Toronto) used here was 13% (weight %) in a 0.01 M phosphate-citrate buffer pH 7.0 25 µl homogenate supernatant of tissue was applied in every slit. There were four slits per gel. The electrophoretic tests were run for 18 hours in a 4° C environment. The current was constant at 15 mA per gel (220x90x7 mm). After the electrophoresis each gel was sliced into two halves, one upper and one lower part. The lower half was incubated with staining solution in total darkness at 20° C for 20 minutes. The staining solution had the following composition:

200 ml 0.2M Tris-buffer, pH 8.6

6 ml 2M Li-lactate (B.D.H. or Sigma)

2.4 ml 30 mg/ml NAD (Sigma)

4 ml 10 mg/ml Nitro-BT (Nitro-Blue-Tetrazolium, Sigma)

1 ml 5 mg/ml PMS (Phenazine Methosulphate, Sigma)

Determination of the activity of LDH-H. In order to determine the activity of LDH-H, it is necessary to suppose that the LDH-isoenzymes have a binomial distribution according to the formula:

$H^4 + 4H^3M + 6H^2M^2 + 4HM^3 + M^4$ = total-LDH-activity.

With this formula and the experimentally determined distribution of activity of isoenzymes, LDH-H and LDH-M have been calculated as per cent activity of total activity according to Palmer and Karlsson (1969) and Karlsson and Palmer (1971). In order to prove that the distribution really is binomial, tests have been made through direct comparison of the observed distribution to the calculated binomial distribution from the values of LDH-H and LDH-M.

Determination of the energy of activation of LDH. In order to determine the energy of activation (µ cal/M), the enzyme-activity was measured according to the above described method at different temperatures from 0° C to 70° C. The energy of activation was calculated between

0° C to 50° C with the Arrhenius equation (Precht et al. 1955):

$$\mu = \frac{4.574 \times \log K_2/K_1}{1/T_1 - 1/T_2}$$

K_1 and K_2 are the activities at the temperatures T_1 and T_2 ($^{\circ}$K).

Results

Heart. See Fig. 11.6.

In hedgehog the LDH-activity is higher during winter and summer than in the guinea-pig.

Liver. See Fig. 11.7.

In hedgehog liver, the LDH-activity is about five times greater than in guinea-pig liver.

White fat. See Fig. 11.8.

During the winter LDH-activity of hedgehog white fat is greater than that in guinea-pigs.

Brown fat. See Fig. 11.9.

The activities of the LDH-isoenzymes from the heart of the hedgehog and the guinea-pig. The distribution of LDH-isoenzymes from hedgehog heart (Fig. 11.10) but not that from guinea-pig heart (Fig. 11.11) follows the binomial distribution. This implies that the figures for guinea-pig LDH-H activity (in % of total) are very uncertain, as these are calculated on the assumption that the distribution is binomial (Fig. 11.12).

The LDH-isoenzymes change during the seasons (Fig. 11.12). Between autumn and winter (I-II) the activity of LDH-H decreases significantly and between spring and summer (III-IV) this activity increases almost significantly (0.05 P 0.10) (Fig. 11.12).

The energy of activation of LDH from the heart of the hedgehog (Fig. 11.13). The energy of activation of LDH from hedgehog heart has significantly ($P = 0.05$) higher values during autumn and winter (I-II) than during spring (III). The increase of energy of activation between

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Fig. 11.6. LDH-activity in units described in the text. The solid line refers to hedgehogs and the interrupted line to guinea-pigs. The number of * denote the significance level according to the "t" test, one * means $0.05 > P > 0.01$ and two ** $P < 0.01$. E.e. refers to the hedgehog (Erinaceus europaeus) and C.p. to the guinea-pig (Cavia porcellus). 1 indicates the difference between seasons I and II, 2 between I and III, 3 between I and IV, 4 between II and III, 5 between II and IV and 6 between III and IV. See further legends Fig. 11.2.

Fig. 11.7. LDH-activity in units described in the text. For legends see Fig. 11.6.

Fig. 11.8. LDH-activity in units described in the text. For legends see Fig. 11.6.

Fig. 11.9. LDH-activity in units described in the text. For legends see Fig. 11.6.

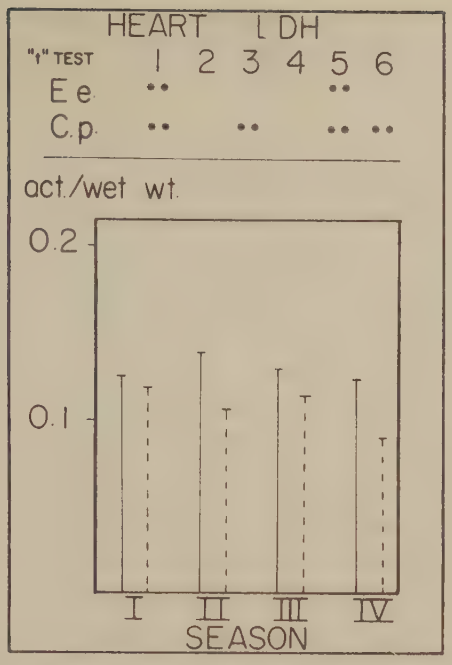
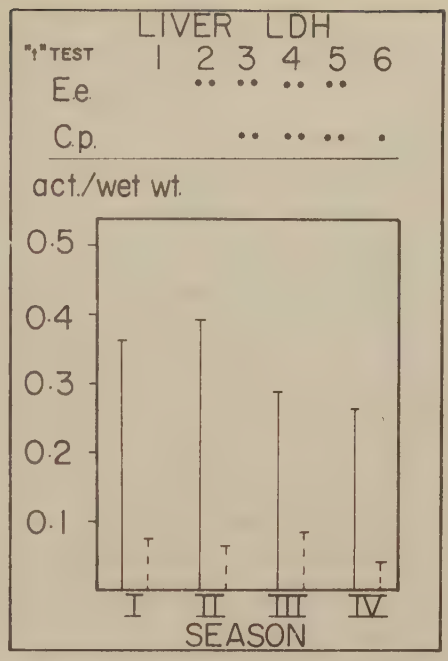


Fig. 11.6



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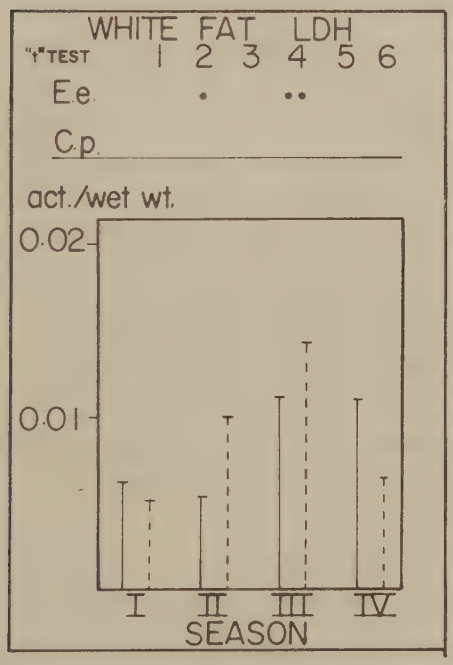

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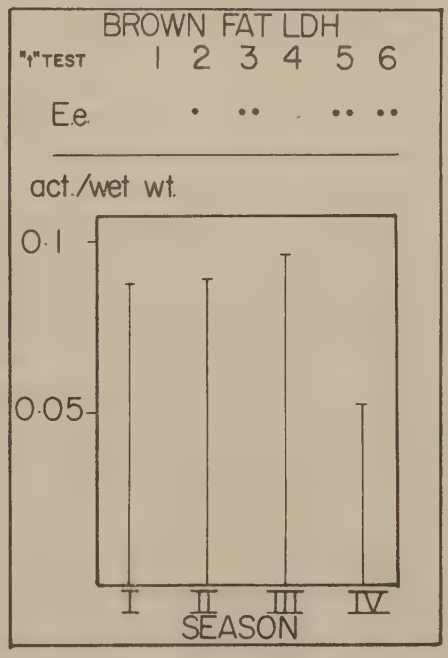


Fig. 11.9

spring and summer (III-IV) is uncertain, as the coefficient of correlation (for linear regression) for summer material is only -0.94.

Discussion

The activity of LDH from hedgehog heart is higher than that of guinea-pig and the former activity increases during hibernation. This may imply that LDH has a great importance during hibernation.

Burlington and Wiebers (1966) have shown that myocardium of hibernators has an increased capacity of anaerobic metabolism as compared to non-hibernators. Hiestrand et al. (1950) have shown that hibernators have an increased resistance to hypoxia in comparison with non-hibernators. Kayser and Malan (1963) and Andjus et al. (1964) have shown that hibernators have an increased capacity for cerebral anaerobic glycosis as compared to non-hibernators. Furthermore, Covino and Hannon (1959) and Hannon et al. (1961) observed that myocardial tissue shows increased capacity for anaerobic metabolism during hibernation. Zimny (1956) found that ATP levels decrease in the myocardium during hibernation and that anaerobic glycolysis is inversely dependent on ATP concentration (Kaplan 1951). In this work Johansson and Senturia (Chapter 6) have described a higher PCO_2, blood standard bicarbonate and a lower pH in hibernating hedgehogs in comparison with the non-hibernating hedgehogs. Popovic (1964) found a decreased arterial oxygen pressure in hibernating ground squirrels in comparison with normothermic animals. If hibernators, with their highly efficient anaerobic metabolism, are hypoxic during hibernation, the concentration of lactate in the blood should increase. Hanson and Johansson (1961) and Twente and Twente (1968) did not find any changes in lactate concentration in any organ. Agid and Ambid (1969) confirmed that the levels of both plasma glucose and lactate are extremely low in the hypothermic garden dormouse, Eliomys quercinus. During arousal the levels of plasma FFA and lactate increase and the amount of oxygen in arterial blood decreases. They suppose that the energy for the first stage of arousal originates from anaerobic glycolysis, as suggested by the increase in lactate.

Fig. 11.10. Percental activity of the LDH-isoenzymes of hedgehog (LDH-1 ◇ , LDH-2 △ , LDH-3 ⬭ , LDH-4 ○ , LDH-5 □). Binomial distribution of the LDH-isoenzymes, based on the calculated LDH-H activity (LDH-1 ◆ , LDH-2 ▲ , LDH-3 ⬬ , LDH-4 ● , LDH-5 ■). For further legends see Fig. 11.6.

Fig. 11.11. Percental activity of the LDH-isoenzymes of guinea-pig. For further legends see Fig. 11.10.

Fig. 11.12. Percental activity of total LDH-H. ♀ hedgehog, ♀ guinea-pig.

Fig. 11.13. Energy of activation for LDH from heart of hedgehog. P = 0.05 in "t" test between either autumn (I) or winter (II) and spring (III).

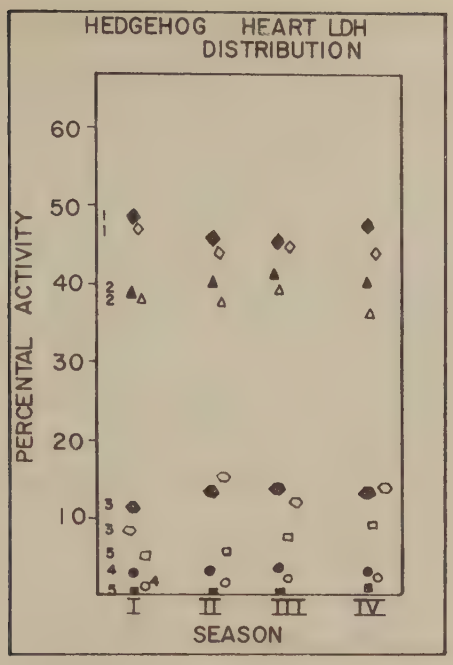


Fig. 11.10

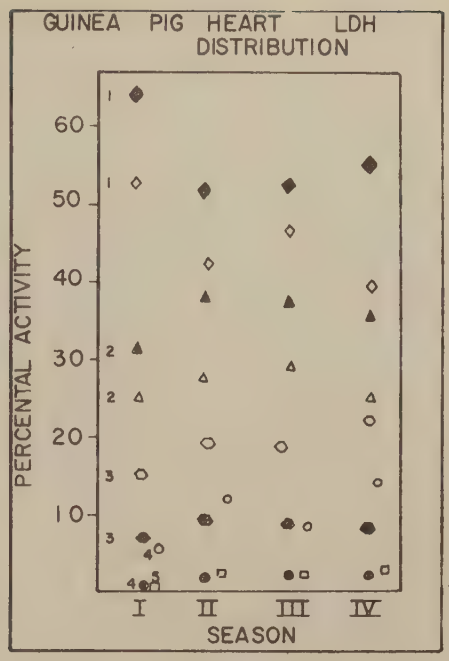


Fig. 11.11

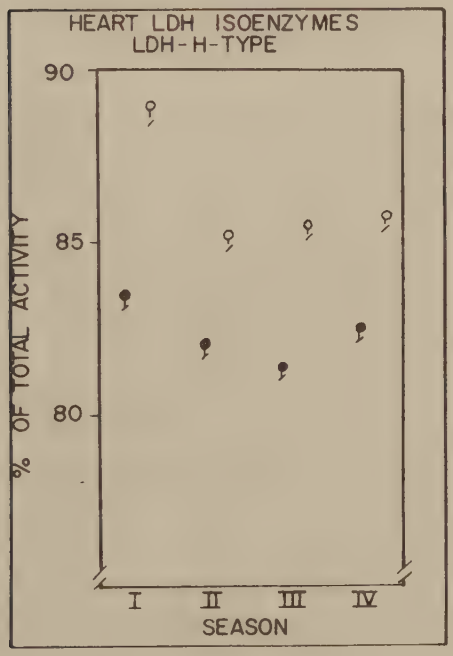


Fig. 11.12

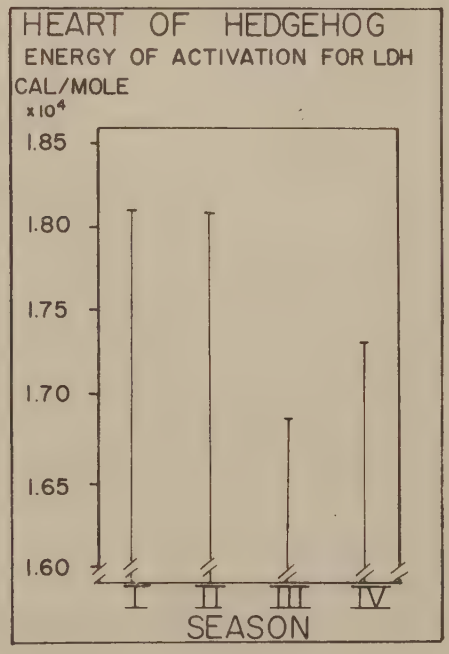


Fig. 11.13

On the other hand, Burlington and Klain (1967) observed that during hypoxia the concentration of lactic acid increases in serum of the ground squirrel (Citellus tridecemlineatus) and simultaneously gluconeogenesis from pyruvate, glycerol, α-ketaglutamate, ℓ-glutamate, ℓ-aspartate and oxaloacetate in kidney cortex slices increases. The rat reacted towards hypoxia, by decreasing gluconeogenesis and by keeping the concentration of lactic acid constant. This shows that the hibernator, as distinguished from the rat, compensates for hypoxia, with anaerobic metabolism and gluconeogenesis. In kidney cortex slices Burlington (1966) has shown that the capacity for gluconeogenesis increases during cold exposure both in rats and in hamsters.

Zimny and Moreland (1968) correlated the low activity of succinic dehydrogenase in heart of the ground squirrel, Citellus tridecemlineatus, with the decrease in the rate of aerobic metabolism during hibernation. Tashima et al. (1970) found that glycolytic intermediates are specifically blocked from entering the Krebs' cycle during hibernation. They also stressed that the hibernator resembles a thiamine-deficient animal with a pyruvate dehydrogenase insufficiency (Weiner and Steyn-Parvé 1959). Chaffee et al. (1961), Denyes and Carter (1961), Denyes and Hassett (1960), South (1960 a and b) and Baumber and Denyes (1963) have all observed an increased capacity of oxidative metabolism in cold acclimated and hibernating hamsters. It is possible that there is a species difference between hamsters (Mesocricetus) and ground squirrels (Citellus) in this respect.

The changes of activities of LDH-H and LDH-M in myocardium (Fig. 11.12) suggest that there is a displacement towards anaerobic conditions during hibernation. This idea is supported by the work of Burlington and Sampson (1968), who found that the activity of LDH-M in myocardium increased during hibernation.

The rat, a non-hibernator, shows decreased activity of LDH-M in myocardium during cold stress (Blatt et al. 1965), which indicates that aerobic metabolism has increased. As noted above the rat is not as capable of anaerobic metabolism as the hibernator (Burlington and Klain 1967). Another non-hibernator, man, increases activity of LDH-M in serum during cold adaptation (Mager et al. 1968). Simmonds et al. (1971) did not find any changes of LDH-isoenzymes in serum of hibernation and non-hibernating ground squirrels, Citellus mexicanus. It is very difficult to correlate these results from different tissues, myocardium and serum. This increase of LDH-M activity in myocardium during hibernation cannot explain the changes of the heat of activation of LDH (Fig. 11.13). Organs with a high activity of LDH-M, for instance liver, have a lower energy of activation than organs with a high activity of LDH-H, for instance heart from hedgehog, the bat (Nyctalus noctula) and guinea-pig (Olsson 1971), which Plagemann et al. (1961) found in rabbits. LDH from the pectoralis muscle of Nyctalus noctula has an even higher value for heat of activation than LDH from the heart of N. noctula. This is due to higher activity of LDH-H in pectoralis muscle than in heart (Olsson 1971). The discrepancy between the results from changes in energy of activation of LDH and from changes of the LDH-H/LDH-M

ratio can of course depend either on methodological errors or thermal changes of the sterical conformation of LDH-2, -3 or -4. Johansson (1969) also showed these changes of the heat of activation and Burlington and Sampson (1968) also showed changes in the LDH-H/LDH-M ratio. The changes in heat of activation of LDH from hedgehog heart are rather small (about 7%) (Fig. 11.13) and should have little importance during hibernation but can reflect greater changes in other enzyme systems. The heats of activation for heart rate of the ground squirrels, Citellus tridecemlineatus and C. mexicanus, are 2.2×10^4 and 2.0×10^4 cal/M respectively (Senturia et al. 1970). The heat of activation for heart rate in hedgehogs is about the same: 2.3×10^4 cal/M, calculated from the values of Kristoffersson and Soivio (1964): 8 beats/min. body temp: 5° C and Björck and Johansson (1955): 181 beats/min. body temp: 32.5° C. It is evident from these values and from the value for the heat of activation for the activity of LDH from hedgehog heart ($1.8 - 1.7 \times 10^4$ cal/M), that LDH cannot be the limiting step in the temperature dependence. The heat of activation of LDH from guinea-pig heart is even lower (1.6×10^4 cal/M) (Olsson 1971).

Liu et al. (1969) found an increased Q_{10}-value for oxygen consumption of liver of hibernating compared to non-hibernating ground squirrels (C. tridecemlineatus), but South (1958, 1960 a and b) found a decreased Q_{10}-value from the hibernating compared to the non-hibernating hamster (Mesocricetus). Whitten and Klain (1968a) did not observe any differences in the Q_{10} of arginase activity or protein synthesis from methionine in liver of hibernating or normothermic Citellus. Further studies on the temperature dependance of essential enzyme systems are needed and will in the future give valuable information about the evolution of homeothermism and hibernation.

The changes of the LDH activity from the heart and the liver of the hedgehog are almost the same, with maximal values during hibernation. These results are supported by the work of Burlington and Klain (1967) and Burlington and Sampson (1968). It might be part of a compensatory mechanism for the low body temperature during the hibernation.

In plasma and erythrocytes of hibernating ground squirrels (Citellus mexicanus) Simmonds et al. (1971) found maximal LDH-activity, but in serum of hedgehogs, guinea-pigs and man, there were no significant changes in LDH-activity between the seasons (Fig. 11.14). There were also no significant seasonal changes in GOT- (Fig. 11.15) or GPT- (Fig. 11.16) activity from serum of hedgehogs, guinea-pigs or man. However, the activity of GPT from summer guinea-pigs is significantly lower than from the other seasons (Fig. 11.16).

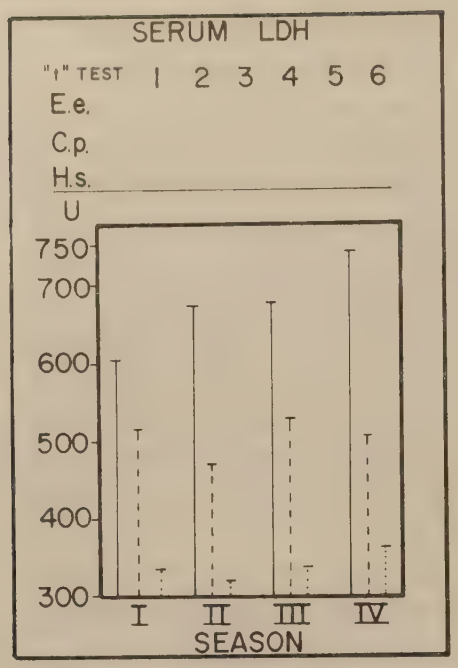


Fig. 11.14

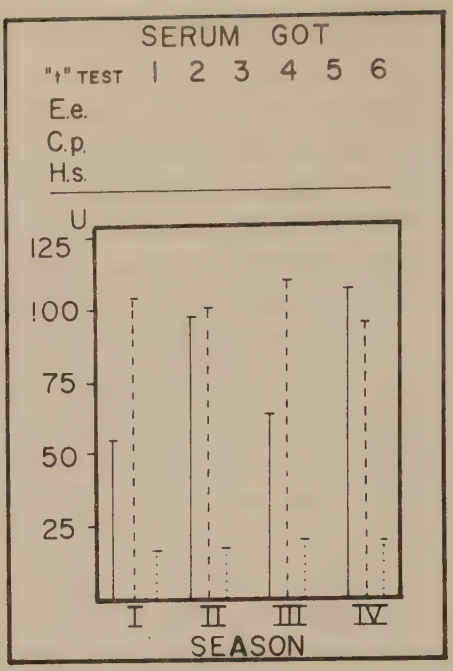


Fig. 11.15

Fig. 11.14. LDH-activity in international units (U) determined by the automatic Technicon method involving NAD fluorimetric determination. For further legends see Fig. 11.6.

Fig. 11.15. Glutamic-Oxaloacetic transaminase (GOT, EC, 2.6.1.1.) activity in international units (U) determined by the automatic Technicon method involving NAD fluorimetric determination. For further legends see Fig. 11.6.

Fig. 11.16. Glutamic-Pyruvic transaminase (GPT, EC, 2.6.1.2) activity in international units (U) determined by the automatic Technicon method involving NAD fluorimetric determination. For further legends see Fig. 11.6.

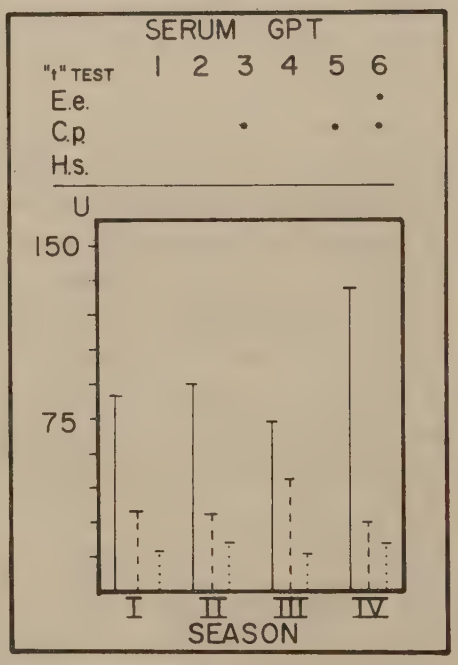


Fig. 11.16

IV. MALIC DEHYDROGENASE. MDH EC 1.1.1.37

Methods

Determination of the activity of the enzyme. The general principles for determination of dehydrogenase activity have been discussed above. The reaction mixture in the spectrophotometric measurement was as follows:

10 µl homogenate supernatant

2.4 ml 0.1M Glycine-buffer, pH 9.8

0.3 ml 0.1M Na malate (B.D.H. or Sigma)

0.3 ml 4.44 mg/ml NAD (Sigma)

The reaction was started by adding the homogenate supernatant to the rest of the reaction mixture.

Determination of the relative activity of the isoenzymes. The technique was exactly the same as the technique previously described for LDH-isoenzymes. The upper half of the starch-gel after electrophoresis was incubated with the following staining solution:

200 ml 0.2M Tris-buffer, pH 8.6

6 ml 2M Na malate

2.4 ml 30 mg/ml NAD

4 ml 10 mg/ml Nitro-BT

1 ml 5 mg/ml PMS

The evaluation of the isoenzyme-bands was the same as for LDH-isoenzymes.

Results

Heart. See Fig. 11.17.

Guinea-pig heart has a greater MDH-activity than hedgehog heart during three seasons (I, III, IV).

Liver. See Fig. 11.18.

There are no significant differences between the activity of hedgehog liver MDH and that of guinea-pig.

White fat. See Fig. 11.19.

During autumn (I), winter (II) and spring (III) the activity of hedgehog MDH is higher than that of guinea-pig.

Brown fat. See Fig. 11.20.

The activities of MDH-isoenzymes from the heart of hedgehog and the guinea-pig (Fig. 11.21). There are small, significant, changes of the relative activity of mitochondrial (M-MDH) and cytoplasmatic (C-MDH) MDH from hedgehog heart. Between hibernation (II) and spring (III) C-MDH increase significantly and between spring (III) and summer (IV) C-MDH decreases

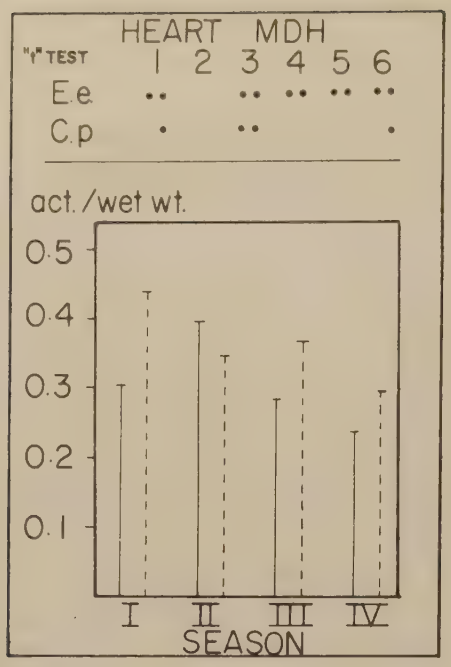
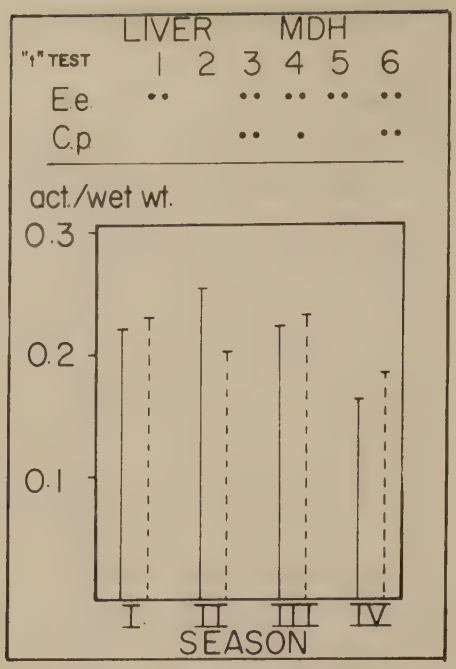


Fig. 11.17



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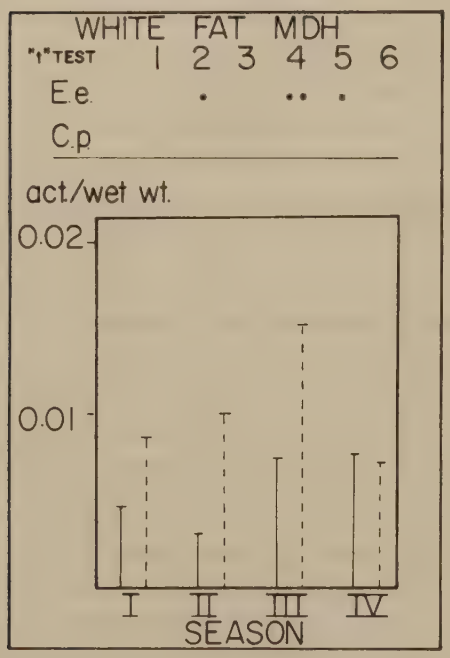

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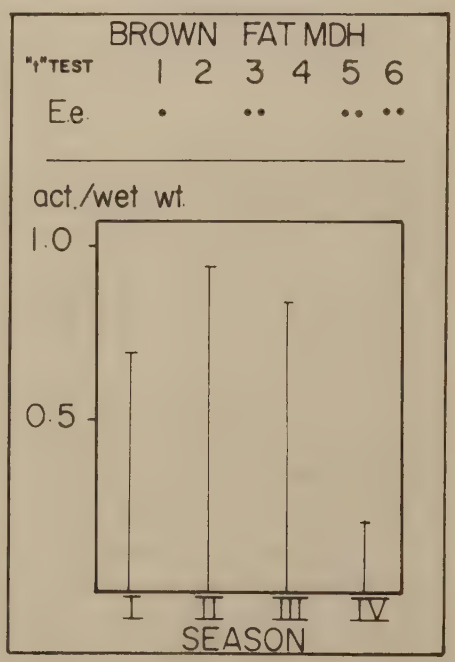

Fig. 11.20

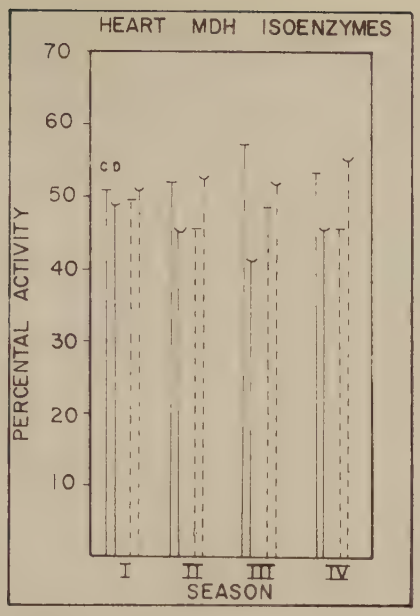

Fig. 11.21

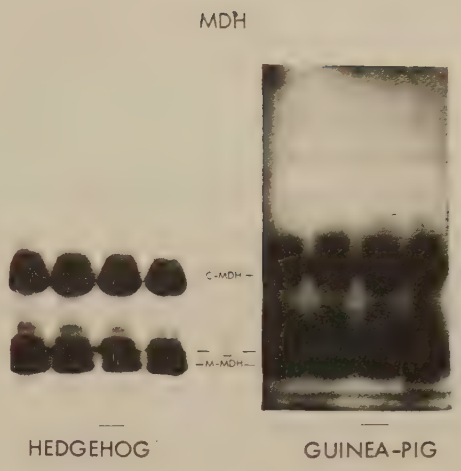

Fig. 11.22

and M-MDH increases significantly.

The MDH-isoenzymes from guinea-pig heart do not change significantly during the year.

Discussion

There is one common feature of the changes of activity of MDH in heart, liver and brown fat from hedgehog; the activity of MDH has a maximum value during hibernation. This argues in favor of either an aerobic metabolism or an increased capacity of gluconeogenesis during hibernation. Burlington and Klain (1967) have shown an increased capacity for gluconeogenesis in tissues of hibernating Citellus tridecemlineatus. Whitten and Klain (1968a) observed an

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Fig. 11.17. MDH-activity in units described in the text. For further legends see Fig. 11.6.

Fig. 11.18. For legends see Fig. 11.17.

Fig. 11.19. For legends see Fig. 11.17.

Fig. 11.20. For legends see Fig. 11.17.

Fig. 11.21. Percental activity of the MDH-isoenzymes of heart: Cytoplasmic (C-MDH) T, Mitochondrial (M-MDH) Y. For further legends see Fig. 11.17.

Fig. 11.22. Electrophoretic separation of the isoenzymes of MDH from the myocardium of a hibernating hedgehog and a guinea-pig from winter.

increased incorporation of alanine into hepatic glycogen during hibernation in Citellus tridecemlineatus. These studies support the concept of increased capacity of gluconeogenesis during hibernation. In slices of kidney cortex, Burlington (1966) has also shown that the capacity for gluconeogenesis increases during cold exposure both in rats and in hamsters. Rebel et al. (1960) observed an increased capacity of C^{14}-acetate incorporation into glycogen in myocardium, brown fat and kidney of hibernating C. citellus compared to the non-hibernating state.

The concentration of glycogen from hedgehog heart and liver increased during the hibernation in this study (Chapter 8). This points to increased gluconeogenesis during hibernation. It has also been shown that the glycogen content of fasting animals increases (Adrouny 1969).

As South and House (1967) pointed out, there is insufficient information to evaluate the results of Rebel et al. (1960).

Acetate can be incorporated into glycogen via malate-NADP-oxidoreductase (EC. 1.1.1.40), oxaloacetate decarboxylase (EC. 4.1.1.3) or phosphoenolpyruvate carboxylase (EC. 4.1.1.32) (Utter and Kurahashi 1954). If the two latter enzymes are of great importance in this process, the activity of MDH should also increase during the hibernation. Whitten and Klain (1968b) have studied the activity of malate-NADP-oxidoreductase from the liver of hibernating and non-hibernating ground squirrels (Citellus tridecemlineatus) and they have found that the activity was considerably lower during hibernation than during June and September.

Rognstad and Katz (1970) have shown that malate and oxaloacetate have great importance in the gluconeogenesis of the rat kidney cortex. From their values it is evident that MDH has twice the capacity of phosphoenolpyruvate carboxylase. This ratio is calculated from the carbon flow from pyruvate in gluconeogenesis. The results of Rognstad and Katz (1970) imply that MDH is involved in the gluconeogenesis from pyruvate, acetate and malate in the tissues studied. Galster and Morrison (1970) reported some interesting data about the glycogen content of the liver and heart of the ground squirrel (Citellus undulatus). In the beginning of each period of hibernation after an arousal period the glycogen content was relatively high but it then declines until next arousal. The glycogen content of the heart was doubled and constant during the winter. Galster and Morrison (1970) concluded that gluconeogenesis in hibernating Citellus undulatus was not sufficient to counteract glycogenolysis. Tashima et al. (1970) proposed from their studies of C^{14}-glucose utilization in C. lateralis, that during hibernation glycolytic intermediates are specifically blocked from entering the Krebs' cycle. Zimny and Moreland (1968) found that the activity of succinic dehydrogenase from the heart of hibernating C. tridecemlineatus decreased to 60% of the non-hibernating value, but Chaffee et al. (1966) found an increased oxidation of succinate by liver mitochondria from hibernating Citellus lateralis. Liu et al. (1969) have also observed a decreased respiratory rate in the liver of hibernating ground squirrel (C. tridecemlineatus) and hamsters (Mesocricetus auratus). In contrast to these results, Frehn (1966) has found an increased succinate-ADP respiration in liver mitochondria from

hibernating chipmunks (Tamias) compared to non-hibernating chipmunks and a decreased succinate-ADP respiration in liver mitochondria from hibernating hamsters compared to non-hibernating hamsters. Liu et al. (1969) suggest that the different responses of succinate respiratory activity to hibernation depend on species differences. Decreased activity of the Krebs' cycle during hibernation has been found in Citellus (Zimney and Moreland 1968) (Tashima et al. 1970) and in Mesocricetus (Liu et al. 1969), but Chaffee et al. (1961), Denyes and Hassett (1960), Denyes and Carter (1961), South (1960 a and b) and Baumber and Denyes (1963) have all observed an increased capacity of oxidative metabolism in cold-acclimated and hibernating hamsters compared to non-hibernating. Increased activity of the Krebs' cycle during hibernation has been found in Tamias (Frehn 1966), Citellus (Chaffee et al. 1966) and Erinaceus (activity of MDH from the heart, liver and brown fat, Figs. 11.17, 11.18, 11.20). There may be differences in the species, season, method and temperature used which may explain the different results. Further studies are necessary on several species in order to solve these problems.

The isoenzymes of MDH: Cytoplasmic MDH (C-MDH) and Mitochondrial MDH (M-MDH) from the heart do not change significantly during the year (Fig. 11.21), except the small changes of the isoenzymes of the hedgehog. These small changes of C-MDH and M-MDH contrast with Moreland's (1962) conclusion that the number of mitochondria increases during the hibernation of ground squirrels, Citellus tridecemlineatus. Zimny and Moreland (1968) correlated this increase of mitochondria to an increased fatty acid oxidation. Poche (1959) found that the number of cristae increased, but the number of mitochondria was constant during the hibernation of the fat dormouse (Glis glis) (See Chapter 15).

The probable importance of equal energies of activation of the two isoenzymes for cold resistance has been proposed by Paulsrud et al. (1970). They found that C-MDH and M-MDH from the brown fat and the liver of the bat (Eptesicus fuscus) have equal energies of activation (15.2 and 14.9 kcal/M). The heat of activation of the mitochondrial-MDH was higher than that of the cytoplasmatic MDH both from the brown fat (16.7 > 13.1 kcal/M) and the liver (17.0 > 12.3 kcal/M) of the rat. They could correlate this low value for the energy of activation of the cytoplasmatic-MDH of the rat with a high electrophoretic mobility. In addition to this Kitto and Wilson (1966) found that C-MDH has lower electrophoretic mobilities from birds capable of natural hibernation. As is seen from Fig. 11.22, there are no differences between the electrophoretic mobilities of C-MDH from the guinea-pig and the hedgehog, but other results (Olsson 1971) show that there is a lower electrophoretic mobility of C-MDH from the bats (Nyctalus noctula and Vespertilio discolor) compared to that from the guinea-pig. Summarizing these results, the theory of Paulsrud et al. (1970) can be relevant for bats but not for hedgehog on the basis of electrophoretic mobility.

Differences between temperature optima for heart MDH from hedgehog and guinea-pigs have been shown (Olsson 1971). The temperature optima of hedgehogs and bats (45° C) lie between that of poikilothermic animals (40° C) and that of homeothermic animals, the guinea-pigs (50° C). There are no changes of MDH whether the hedgehog is hibernating or not, neither are there any great differences in the energy of activation from the heart of guinea-pig, hedgehog or bat. These results show that there have been adaptive changes of MDH in the evolution of homeothermic and hibernating mammals and this is quite in agreement with Johansson's (1969b) observations on LDH and cholinesterase.

During the hibernation, aerobic metabolism in hedgehog white fat is significantly reduced as shown in the decrease of MDH activity.

Brown fat has a high capacity for aerobic metabolism and thus exhibits a high activity of MDH. This activity is higher during hibernation and posthibernation. During these periods the brown fat is hyperactive in the arousal processes. Hedgehog brown fat has a thermogenic effect during arousal, even in a warm environment (Edwards and Munday 1969a). Draskoczy and Lyman (1965) and Edwards and Munday (1969a) have observed that brown fat is active during hibernation. This is in agreement with the high activity of MDH during hibernation and posthibernation. Edwards and Munday (1969a) noticed that it was impossible to relate the activity of the brown fat during hibernation with heat production, since the temperature of brown fat and other areas could not be recorded without initiating arousals. Hibernation is naturally interrupted by several arousals (Kristoffersson and Soivio 1964, Galster and Morrison 1970).

In this study the period of posthibernation is very close to hibernation, as some of the hedgehogs from the posthibernation period in fact were still hibernating (see above, Chapter 2).

V. GLUCOSE-6-PHOSPHATE DEHYDROGENASE, G-6-PDH EC 1.1.1.49.

Methods

The general principles for the determination of dehydrogenase activity have been discussed above. The reaction mixture in the spectrophotometric measurement was as follows:

100 µl supernatant of the homogenate

2.6 ml 0.1M Tris-Buffer, pH 8.0

0.1 ml 0.03M Glucose-6-phosphate (Sigma)

0.1 ml 0.01M NADP (Sigma)

0.2 ml 0.15M $MgCl_2$

The reaction was started by adding the supernatant of the homogenate to the rest of the reaction mixture.

Results

Heart. See Fig. 11.23.

The activity from hedgehog heart is twice as high as that from guinea-pig heart.

Liver. See Fig. 11.24.

The activity from hedgehog liver is twice that of guinea-pig liver.

White fat. See Fig. 11.25.

The activity of G-6-PDH from the guinea-pig white fat is almost ten times that of the hedgehog.

Brown fat. See Fig. 11.26.

Discussion

The activity of G-6-PDH from hedgehog heart and liver decreases after hibernation and seems to increase just before hibernation. Teleologically, the activity of G-6-PDH appears not to be as important for hedgehog heart as for that of the guinea-pig, since the activity is higher in guinea-pig heart. Furthermore, the activity of G-6-PDH is 8 times as high in liver as in heart of the hedgehog and only twice as high in the same organs of the guinea-pig.

The ground squirrel (Citellus undulatus) has 18 and the rat 4 times higher G-6-PDH activity in liver than in heart (Hannon and Vaughan 1961). This suggests that G-6-PDH has a relatively greater importance for the liver of the hibernator than for liver of the non-hibernator and vice versa for the heart.

Hannon and Vaughan (1961) also found a small increase of the activity of G-6-PDH from the heart of hibernating ground squirrels, Citellus undulatus, which compared to non-hibernating ground squirrels supports the above mentioned changes for the heart of the

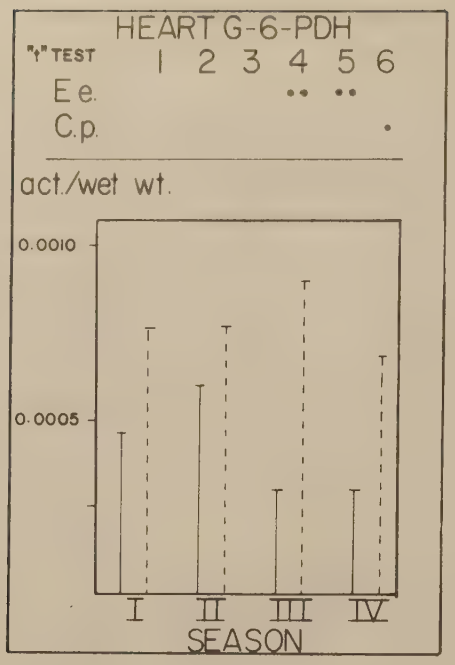
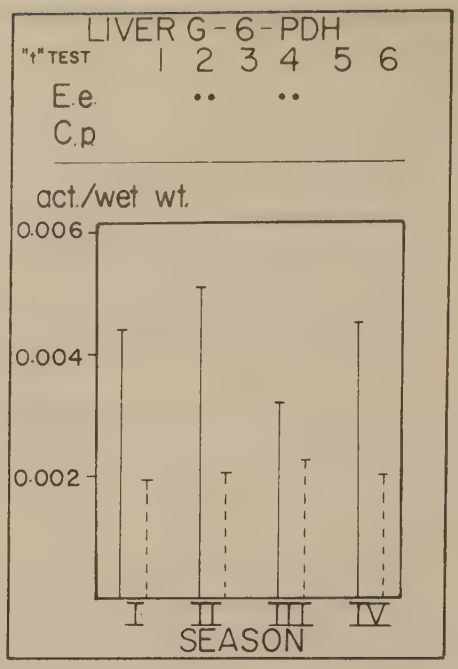


Fig. 11.23



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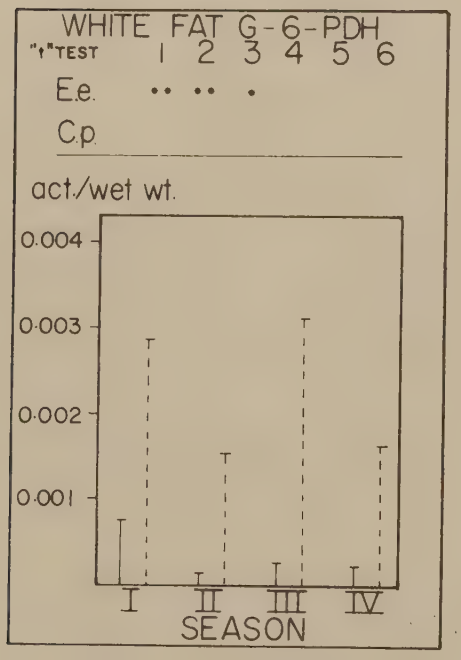

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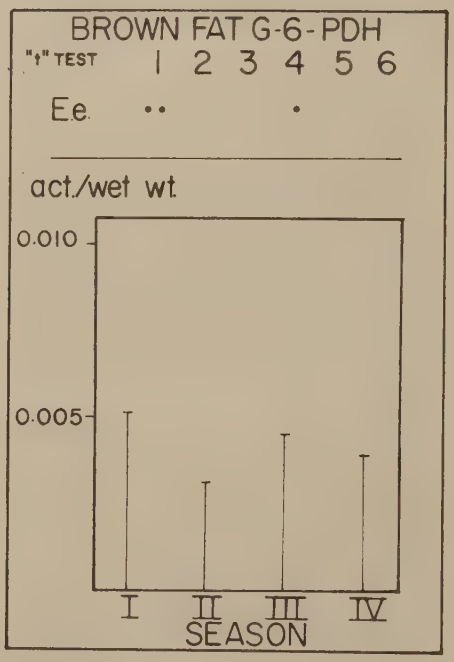

Fig. 11.26

hedgehog. Hawley (1968) has shown that the oxygen uptake in vitro at 35° C is greater in the heart of the ground squirrel, (C. tridecemlineatus) when hibernating than when not hibernating in the presence of G-6-P, NAD(H) of NADP(H) and F. This shows that the pentose-shunt has a greater importance during hibernation than in the aroused state, which is in agreement with the high activity of G-6-PDH from the heart and the liver of the hibernating hedgehog. Hawley's work (1968) suggests that the hibernating heart is more dependent on fatty acid catabolism than the non-hibernating heart.

G-6-PDH has a great importance for the synthesis of fatty acids and steroids as $NADPH_2$ is formed at the oxidation of G-6-P (Tepperman and Tepperman 1958, 1961, South and House 1967). It is important in this discussion to remember that $NADPH_2$ also can be formed by other processes (Lowenstein 1961, Flatt and Ball 1964, Pande et al. 1964 and Kornacker and Ball 1965).

Forsberg and Sarajas (1955) found a higher total liver lipid content and an increased incorporation of glucose into the liver lipids in hibernating hedgehogs (Chapter 9). This is in agreement with the high G-6-PDH activity of the liver of the hibernating hedgehog, but this is not supported by Hannon and Vaughan (1961), who found that the activity of G-6-PDH from the liver of ground squirrels (Citellus undulatus) decreased during the hibernation. Whitten and Klain (1968b) also reported a decreased incorporation of glucose and acetate into lipids and a decrease of G-6-PDH activity in the liver of hibernating ground squirrels (Citellus tridecemlineatus). It is quite possible that there are great species differences in the lipogenesis during hibernation. Tashima et al. (1970) suggest from their studies of C^{14}-incorporation that lipogenesis is profoundly depressed in the heart, the liver, the white and brown fat of ground squirrels (Citellus lateralis) during hibernation. Denyes and Carter (1961) showed that there is a decrease of lipogenesis in the liver of hamsters (Mesocricetus) during the hibernation. Baumber and Denyes (1963) found an increase of lipogenesis in the epididymal fat during hibernation in hamsters (Mesocricetus). It is evident that there is a lipogenic difference between these two organs of hamsters (Mesocricetus). It seems as if the liver and the white fat of the hedgehog behave in an opposite way. Lipogenesis in hedgehog white fat may be decreasing during hibernation as is seen from the striking decrease of the activity of G-6-PDH. It is well-known that lipogenesis

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Fig. 11.23. G-6-PDH-activity in units described in the text. For further legends see Fig. 11.6.

Fig. 11.24. For legends see Fig. 11.23.

Fig. 11.25. For legends see Fig. 11.23.

Fig. 11.26. For legends see Fig. 11.23.

is high during prehibernation (Kayser 1961, Smalley and Dryer 1967). Thus this decrease in the activity of G-6-PDH from the white fat between prehibernation and hibernation can be seen as a consequence of the high lipogenic activity during the prehibernation period.

The changes in activity of G-6-PDH from the brown fat of the hedgehog are almost the same as from the white fat with the exception that the activity from the brown fat increased even after hibernation. This indicates that the lipogenesis in this tissue starts almost at once after hibernation (Chapter 15). Tashima et al. (1970) found an accelerated incorporation of glucose into total lipids and especially glyceride-glycerol in the brown fat during the first hour of arousal in ground squirrels (Citellus lateralis).

VI. α-GLYCEROPHOSPHATE DEHYDROGENASE, α-GPDH EC 1.1.1.8.

Methods

The general principles for determination of dehydrogenase activity have already been discussed.

The reaction mixture in the spectrophotometric measurement was as follows:

100 µl homogenate supernatant

2.4 ml 0.1M Glycine-buffer, pH 9.22

0.3 ml 0.1M α-Glycerolphosphate (Sigma)

0.3 ml 4.44 mg/ml NAD (Sigma)

The reaction was started by adding the supernatant of the homogenate to the rest of the reaction mixture.

Results

Heart. See Fig. 11.27.

The activity of α-GPDH from hedgehog heart is significantly higher than that of the guinea-pig during all seasons except the autumn (I).

Liver. See Fig. 11.28.

The activity of α-GPDH from guinea-pig liver is significantly higher than that of the hedgehog except during the autumn (I).

White fat. See Fig. 11.29.

During winter and spring the activity of α-GPDH from white fat is higher from guinea-pig than from hedgehog.

Brown fat. See Fig. 11.30.

Discussion

α-GPDH from hedgehog heart has a significantly higher activity than from guinea-pig heart except for autumn. Bücher and Klingenberg (1958) and Sacktor (1959) showed that there are both extra- and intramitochondrial forms of α-GPDH. The intramitochondrial form oxidizes α-GP to dihydroxyacetonephosphate, which the extramitochondrial form reduces to α-GP. Chaffee et al. (1964), Chaffee et al. (1966) and Dryer and Paulsryd (1966) have emphasized the importance of this process for the gain of energy from glycerol from fat catabolism. During hydrolysis of fats glycerol is formed, which is phosphorylated to α-GP, which then can be utilized in glycolysis (Chaffee et al. 1964, Dryer and Paulsryd 1966) by oxidation.

The low activity of α-GPDH from hedgehog heart during autumn can be correlated to the low fat catabolism during that season. The significantly higher activity of α-GPDH from

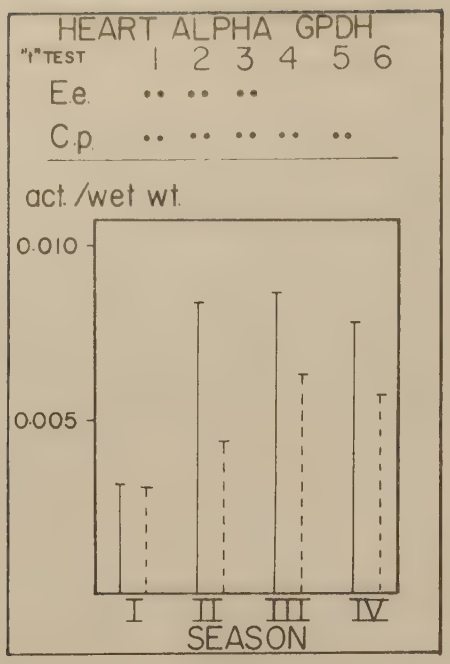

Fig. 11.27

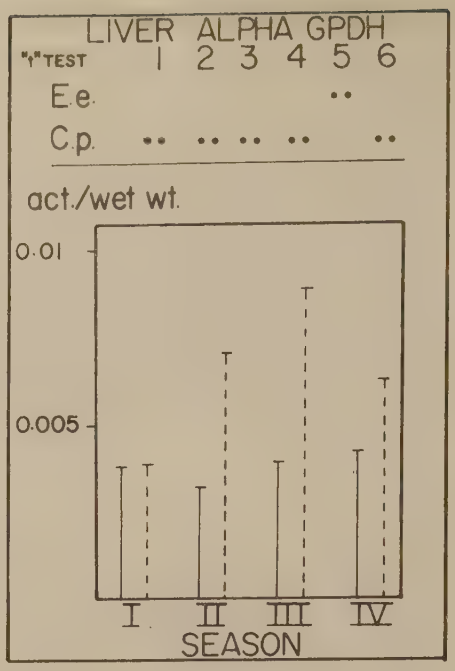

Fig. 11.28

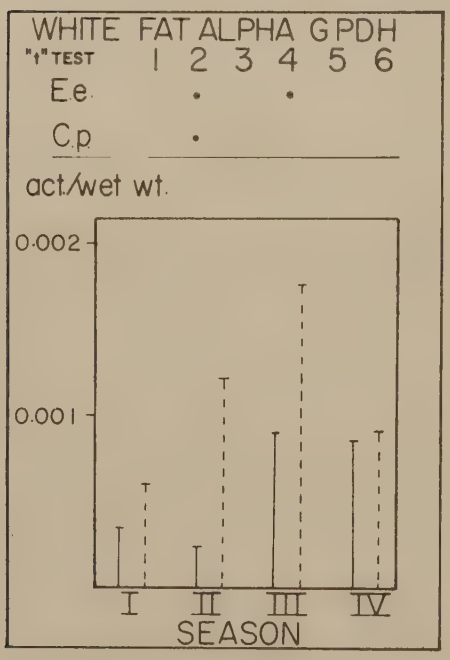

Fig. 11.29

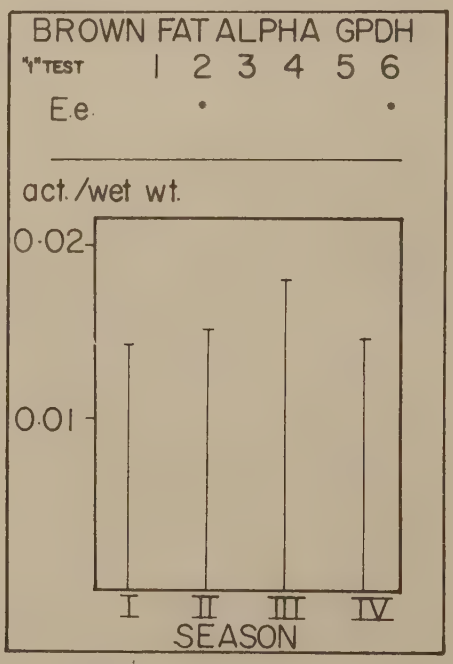

Fig. 11.30

hedgehog heart and the increase in activity during hibernation and the decrease after the spring, can imply that hedgehog heart uses glycerol phosphate as an energy pool to a greater extent during hibernation in comparison with the prehibernatory state and guinea-pig heart.

The activity of α-GPDH is higher in the liver than in the heart of the guinea-pig. The increase in activity of α-GPDH from hedgehog liver between hibernation and post-hibernation is in agreement with the high lipolysis seen during the spring. The low activity of α-GPDH during hibernation supports the results of Chaffee et al. (1966), who found a low α-GPDH activity in the liver mitochondria of hibernating ground squirrels (Citellus lateralis).

The significant increase of the activity of α-GPDH from hedgehog white fat between winter and spring can be an expression of the high lipolytic activity during the spring. The serum glyceride-glycerol concentration of the hibernating hedgehog was low but it was higher when arousing (Konttinen et al. 1964). The same tendency as seen in white fat is quite obvious in the changes of α-GPDH from the hedgehog brown fat. In this tissue there is an increase in activity before spring and a decrease after spring. This is correlated to a high rate of lipolysis during arousals from hibernation and the decrease in lipolysis rate after arousals. Joel (1965) found a 50% decrease in lipid concentration in recently aroused ground squirrels (Citellus tridecemlineatus) in comparison with the concentration just before arousal. This great release of lipids and glycerol can be utilized by other organs.

Hardman and Hull (1970) concluded from their studies on newborn rabbits "that brown adipose tissue releases significant amounts of fatty acid and glycerol into the circulation and that this contribution is greatly increased with noradrenalin infusion and presumably cold exposure and that brown adipose tissue depleted of fat produces heat by drawing free fatty acids as well as glucoses from the circulation". Horwitz and Nelson (1968) agree with these conclusions. Prusiner et al. (1968) proposed that most of the energy for nonshivering thermogenesis comes from fatty acid oxidation. In this connection it is important not to overlook the calorigenic potential, which arises from the oxidation of

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Fig. 11.27. α-GPDH-activity in units described in the text. For further legends see Fig. 11.6.

Fig. 11.28. For legends see Fig. 11.27.

Fig. 11.29. For legends see Fig. 11.27.

Fig. 11.30. For legends see Fig. 11.27.

α-GP (Green 1936, Bücher and Klingenberg 1958, Estabrook and Sacktor 1958, Smith 1964). Brown fat has the greatest activity of α-GPDH in the organs studied, which supports the observation by Chaffee et al. (1966) that in ground squirrels (Citellus lateralis) as in hamsters (Mesocricetus) (Chaffee et al. 1961) the respiration of mitochondria on α-GP was low in systems from the liver but relatively high in those from brown fat.

Prusiner et al. (1970) reviewed the mechanisms controlling oxidative metabolism in brown fat and mentioned that α-GP as a substrate as well as succinate and fatty acids + carnitine stimulated oxidation both in mitochondria and in cells of brown fat. Thus α-GP may be important for brown fat both as a trigger substance and as a calorigenic substance. As mentioned above the activity of α-GPDH is high in brown fat and thus brown fat can oxidize α-GP.

Chaffee et al. (1966) found a maximum for α-GPDH activity from brown fat during hibernation of ground squirrels (Citellus lateralis). The activity of α-GPDH from hedgehog brown fat had its maximum value during spring, the arousal season.

VII. SUMMARIZING DISCUSSION

The seasonal changes of the activities of the four dehydrogenases LDH, MDH, G-6-PDH and α-GPDH from heart, liver, white and brown fat of hedgehog have one common feature. In all organs, except white fat, all enzymes have maximal activity during hibernation. However, G-6-PDH in brown fat and α-GPDH in brown fat and α-GPDH in liver are exceptions. Seasonal changes in activities of these four enzymes from the same organs of guinea-pig are quite different from that of hedgehog. Only α-GPDH from hedgehog and guinea-pig heart show similar changes.

The activities of LDH and α-GPDH in heart and G-6-PDH in liver from hedgehog are significantly higher than the corresponding activities from guinea-pig. On the other hand the activities of MDH and G-6-PDH in heart, α-GPDH in liver and MDH, G-6-PDH and α-GPDH in white fat from guinea-pig are significantly higher than the activities from hedgehog. These results may indicate the following differences between the hedgehog and guinea-pig:

1. a higher anaerobic capacity for heart and liver of hedgehog than guinea-pig (see discussion below and in the section on LDH above).

2. a higher capacity for the pentose-shunt in hedgehog liver and in guinea-pig heart and white fat than other tissues examined (see discussion in the section on G-6-PDH).

3. a higher capacity for glycerol metabolism in hedgehog heart and in liver and white fat of guinea-pig than in other tissues examined (see discussion in the section on α-GDPH).

It may not be meaningful to compare seasonal changes of enzyme activities from the hedgehog, a hibernator, with those from the guinea-pig, a non-hibernator, since the two species did not live under identical environmental conditions in this study (see Chapter 2 above). In addition the two species belong to different mammalian orders, Insectivora and Rodentia.

Some information about the ratio of anaerobic metabolism may be obtained by the enzyme activity quotient Q LDH/MDH, which was proposed by Bücher and Klingenberg (1958). When applying the quotient to hedgehog and guinea-pig organs (see Table 11.I) it is evident that the hearts of both species have a dominant aerobic metabolism as does guinea-pig liver. This is in agreement with van den Hende et al. (1968), who have studied Q LDH/MDH in the same organs from pigs. It is remarkable that the quotient from hedgehog liver is so high. This supports the observation that hibernators have a high anaerobic capacity (see section on LDH above). The quotient is also a little higher from hedgehog white fat than from guinea-pig white fat. Hedgehog brown adipose tissue has an extremely low quotient and it is well-known that this organ has a very high aerobic metabolism (Lindberg ed. 1970).

It has been stated that hibernators are more capable of anaerobic metabolism than non-hibernators. This study lends support to this opinion. One indication is that the quotient of LDH/MDH for liver and white fat are higher in hedgehog than in guinea-pig irrespective of season. Another indication is the higher activity of LDH-H from guinea-pig heart as

Table 11.1.

Ratio of the mean activities of LDH and MDH (Q LDH/MDH).

		I	II	III	IV
Heart	Hedgehog	0.41	0.35	0.46	0.54
Heart	Guinea-pig	0.27	0.31	0.31	0.30
Liver	Hedgehog	1.6	1.5	1.3	1.6
Liver	Guinea-pig	0.33	0.34	0.35	0.24
White fat	Hedgehog	1.2	1.7	1.5	1.4
White fat	Guinea-pig	0.56	0.91	0.93	1.0
Brown fat	Hedgehog	0.13	0.097	0.12	0.25

compared to hedgehog. The latter conclusion is questionable because the LDH-isoenzymes from guinea-pig heart do not show a strict binomial distribution.

The lower activity of LDH-H from heart of the hibernating hedgehog as compared with the non-hibernating hedgehog should indicate decreased capacity for aerobic metabolism during hibernation. However, the LDH/MDH quotients do not differ between hibernating and non-hibernating hedgehogs.

The increased activity of MDH during hibernation of the hedgehog can either be an expression for increased aerobic capacity or increased capacity of gluconeogenesis, which is discussed in the section on MDH. In light of the results from LDH and Q LDH/MDH, it is probable that the increased activity of MDH represents an increased capacity for gluconeogenesis in hedgehog heart and liver. On the other hand, brown adipose tissue with a very high aerobic metabolism increased its aerobic capacity further during hibernation by increasing MDH-activity. White adipose tissue, with a dominant anaerobic metabolism reacted in the opposite way during hibernation.

The importance of the pentose-shunt is to some extent the synthesis of $NADPH_2$, which is discussed in the section on G-6-PDH. The activity of G-6-PDH, which is the first enzyme in the pentose-shunt decreases after hibernation in hedgehog heart and liver and increases slightly before hibernation. This seems to be an expression for high lipogenesis in heart during hibernation and a lower one after hibernation. Lipogenesis in white and brown fat is maximal in autumn, as is seen from the maximal values of G-6-PDH activity in autumn. In addition, the activity of G-6-PDH in brown fat increases after hibernation.

The activity of α-GPDH is a reflection of glycerol turnover and thus the rate of metabolism of lipids. It appears that hedgehog heart has a high capacity for the turnover of lipids, especially during hibernation and spring. This is supported by the high activity of G-6-PDH in

heart during hibernation. The adipose tissues, both white and brown, show maximal α-GPDH-activity during spring, the arousal season.

The most interesting result from the changes of soluble proteins is the maximal values during hibernation and posthibernation in brown fat of hedgehog. This may be correlated to the large number of mitochondria during hibernation and posthibernation (see Chapter 15). The mitochondrial changes are correlated to the high aerobic metabolism in this tissue.

Chapter 15

Ultrastructure of Brown Adipose Tissue and Myocardium

by

Sven-Olle R. Olsson

The following questions are of interest in this seasonal study of hedgehogs and guinea-pigs:

1. Are there any seasonal changes in the ultrastructure of brown adipose tissue of hedgehogs ? Reviews have been published by Rasmussen (1923), Johansson (1959) and Lindberg (1970).

2. Are there any ultrastructural differences between the myocardium of hedgehogs and guinea-pigs? Such differences could help to explain the differences in temperature tolerance between the myocardium of hibernators and non-hibernators (Johansson 1967, 1969 a and b and Chapter 11).

3. Are there any adaptive ultrastructural differences in the myocardium between hibernating or non-hibernating hedgehogs? Such differences have been reported from the ground squirrel (Citellus tridecemlineatus) by Rosenquist and Zimny (1969) and Rosenquist (1970), but opposing results have been found from the ground squirrel (Citellus lateralis) by Aloia and Pengelley (1971).

Method

The apex of the left ventricle was excised from four hibernating (II) and four non-hibernating (IV) hedgehogs and from four guinea-pigs during winter (II) and four during summer (IV). (See General Methods and Materials, Chapter 2, Fig. 2.1.) The piece of myocardium was cut into 1 mm cubes. From the same hedgehogs, pieces of brown adipose tissue were excised and also cut into 1 mm cubes.

The pieces of tissue were fixed in 3% glutaraldehyde in a phosphate buffer (Millonig 1961), washed in buffer, postfixed in 1% osmium tetroxide in a phosphate buffer (Millonig 1961), dehydrated in ethanol, block stained in 1/2% uranyl acetate and 1% phosphotungstic acid in ethanol, and embedded in Vestopal W® (Martin Jaeger, Geneva, Switzerland). The embedded tissues were sectioned and then stained with 1% uranyl acetate and 0.1 g/100 ml

lead citrate[1]. The sections were studied with a Philips EM 300 and a Hitachi HS-7S.

Results

Brown adipose tissue. No differences in the shape of size of nuclei were found from hibernating and non-hibernating hedgehogs. The size of the nucleus is 4-6 μ.

A greater number of mitochondria per adipocyte were observed in brown fat from hibernating hedgehogs (Fig. 15.1 b) as compared to non-hibernating (Fig. 15.1 a).

The shape of the mitochondria is polygonal from hibernating and almost circular from non-hibernating hedgehog. The arrangement of cristae from mitochondria from both seasons is long, slender, gently curved and non-branching cristae reaching from one side of the mitochondrion to the other.

There are no significant differences of the sizes of mitochondria (1-2 μ) from hibernating or non-hibernating hedgehogs.

The number of fat droplets per adipocyte was greater in tissue from hibernating hedgehog than from non-hibernating. However, the fat droplets were larger from non-hibernating (5-10 μ) compared to hibernating hedgehogs (1-5 μ). It is quite obvious that the amount of fat per adipocyte is significantly greater from the non-hibernating hedgehog compared to the hibernating hedgehog (Fig. 15.1).

The shape of the fat droplets is always spherical.

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1. a) 1% uranyl acetate in 75% ethanol 20 min at 20° C.
   b) Washed in 50% ethanol and aq.dest. and dried.
   c) Lead citrate 0.1 g/100 ml 0.1N NaOH 2 min at 20° C.
   d) Washed in distilled water.

Fig. 15.1.a. Brown adipose tissue from summer hedgehog (IV). Big fat droplets (F) occupy most of the all volume. The number of mitochondria (M) is small. Magnification 6 000 x.

Fig.15.1.b. Brown adipose tissue from hibernating hedgehog (II). The fat droplets (F) are smaller but more numerous and the number of mitochondria (M) is greater than from non-hibernating hedgehog. Magnification 6 000 x.

Fig. 15.1.c. Brown adipose tissue from hibernating hedgehog (II) showing pinocytosis (P). Magnification 19 000 x.

Fig. 15.1.d. Brown adipose tissue from summer hedgehog (IV) showing pinocytosis (P). Magnification 19 000 x.

Fig. 15.1.e. Brown adipose tissue from hibernating hedgehog (II) showing a developing brown adipocyte with an obvious sarcoplastic reticulum (SR). Magnification 19 000 x. The line below the micrographs represents 1 μm.

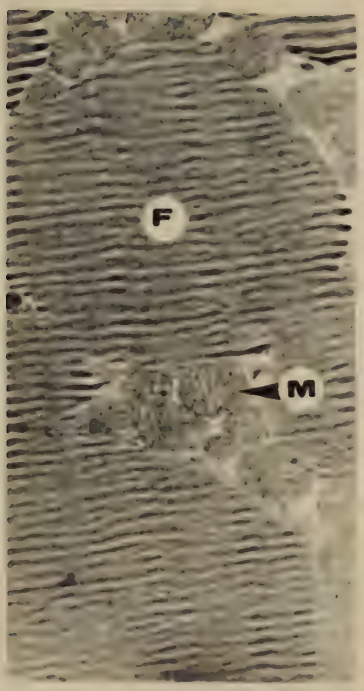

Fig. 15.1a 1 µm.

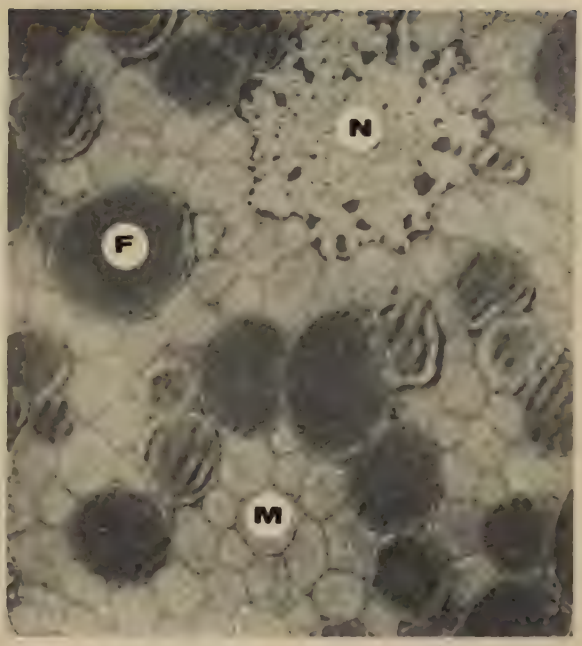

Fig. 15.1b 1µm

Fig. 15.1c 1 µm

1 µm

Fig. 15.1d

Fig. 15.1e 1 µm

The sarcolemma has a rather smooth structure, although pinocytosis has been observed both from hibernating (Fig. 15.1.c) and non-hibernating (Fig. 15.1.d) hedgehog.

The sarcoplasmic reticulum can hardly be seen from hibernating hedgehog, but is more prominent from the non-hibernating hedgehog. A well developed sarcoplasmic reticulum has been found in a developing adipocyte (Fig. 15.1.e) from hibernating hedgehog.

Heart. There were no apparent differences in the structure of the nuclei from myocardium of hibernating and non-hibernating hedgehogs. For both seasonal stages the contours of the nuclei were rather smooth (Fig. 15.2.a) with a distinct double layed nuclear membrane and with osmiophilic material concentrated to the periphery. However, the nuclei of the myocardium from guinea-pig were more irregular (Fig. 15.2.c) as compared with the hedgehog. In some nuclei a nucleolus was visible.

No obvious differences in the number of mitochondria from hibernating and non-hibernating hedgehog and guinea-pig have been found.

The shape of the mitochondria are either circular or elliptical, depending on the direction of section. No typical long slender mitochondria have been found. The cristae are usually long, thin folds of the inner mitochondrial membrane, reaching from one side of the mitochondrion to the other. No branching of the cristae has been observed. There are no differences in the shape or number of cristae in the mitochondria from hibernating (Fig. 15.3.b) or non-hibernating hedgehog (Fig. 15.3.a) or guinea-pig (Fig. 15.3.d). The number of cristae per mitochondrion is 20-30. Fig. 15.3.b is an exception with about 50 cristae. The thickness of the cristae varies between 10-20 mμ, no significant difference between the species or seasons can be observed. Some special mitochondria have been observed from a hibernating hedgehog (Fig. 15.3.c) and a guinea-pig (Fig. 15.2.c). These giant mitochondria (Fig. 15.3.c)

Fig. 15.2.a. Heart muscle cell from hibernating hedgehog (II) showing a part of a typical nucleus (N) with a nucleolus (NL) and some pinocytosis (P). FI = muscle fiber. Magnification 19 000 x.

Fig. 15.2.b. Heart muscle cell from summer hedgehog (IV) showing the typical zig-zac manner of the sarcolemma (S) at each Z-line of the muscle fiber (FI). Magnification 5 000 x.

Fig. 15.2.c. Heart muscle cell from guinea-pig (IV) showing a typical nucleus (N), the zig-zac manner of the sarcolemma (S), the muscle fibers (FI) and a strange form of mitochondria (M) with circular concentric cristae. Magnification 6 000 x.

The line below the micrographs represents 1 μm.

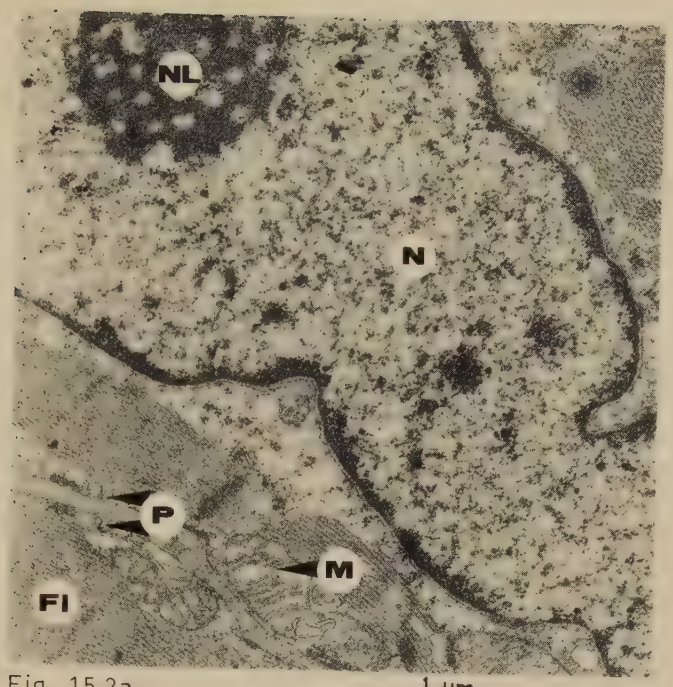

Fig. 15.2a

1 µm

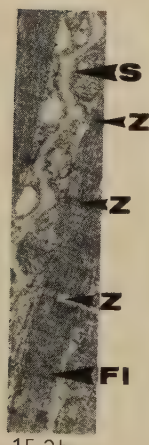

Fig. 15.2b

1µm

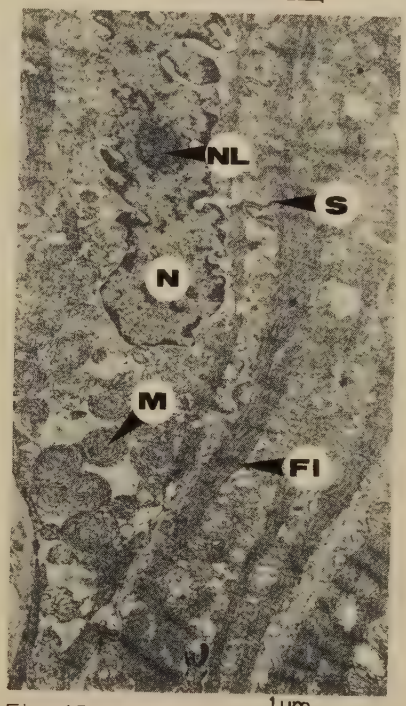

Fig. 15.2c

1µm

from one hibernating hedgehog are about 3-4 μ with a large number of densely packed cristae. The cristae seems to be a little curved and non-branching and seems to cross the whole width of the mitochondrion. These giant mitochondria are not typical for hibernating hedgehogs, as they have been found in only one hedgehog and not in guinea-pigs. The special mitochondria from the guinea-pig (Fig. 15.2.c) are of normal size, but the cristae are arranged in a circular manner.

No significant difference in the size of mitochondria between the species or seasons can be osberved. The size of the mitochondria varies between 0.5-2 μ.

The intercalated discs are well developed in the myocardium of hedgehog and guinea-pig. The continuity between sarcolemma and the intercalated discs is illustrated in Fig. 15.4.a and 15.4.b. The different structures of the intercalated disc (Dewey 1969) are seen in the direction of the muscle fibers in Fig. 15.4.a and in a direction perpendicular to the muscle fibers in Fig. 15.4.b. All these three components of the intercalated disc have a normal mammalian appearance. There are no differences between guinea-pig and hibernating and non-hibernating hedgehog. The intercellular space in the disc is about 2 mμ.

The sarcolemma has a very special configuration in myocardium. At each Z-line of the myofibrills there is an indentation of the sarcolemma to make the distance between the sarcolemma and the Z-line of the myofibrills as short as possible. This is illustrated in Fig. 15.2.b and c. It is evident from this study that the frequency of pinocytosis is rather great. There are no differences between guinea-pig, hibernating or non-hibernating hedgehog in these respects.

The two components of the sarcoplasmic reticulum are about equally developed in the two species and no differences between hibernating and non-hibernating hedgehog have been found. The transverse system (TS) is easily seen close to the Z-lines in Fig. 15.4.b, 15.5.a, and 15.5.b. The longitudinal system can be seen in Fig. 15.4.b and 15.5.a. The internal coupling

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Fig. 15.3.a. A typical mitochondria from summer hedgehog (IV) heart. Magnification 32 000 x.

Fig. 15.3.b. A mitochondria with an unusual great number of cristae from hibernating hedgehog heart. Magnification 32 000 x.

Fig. 15.3.c. Heart muscle cell from hibernating hedgehog (II) with enormous great mitochondria with a large number of cristae. Magnification 6 600 x.

Fig. 15.3.d. A typical mitochondria from guinea-pig heart. Magnification 32 000 x.
The line below the micrographs represents 1 μm.

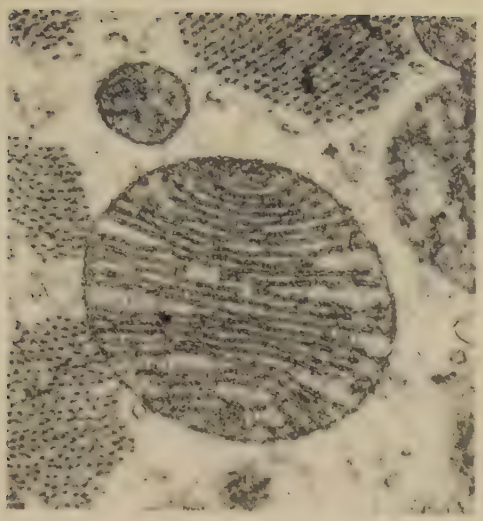


Fig. 15.3a 1 µm

Fig. 15.3b 1 µm

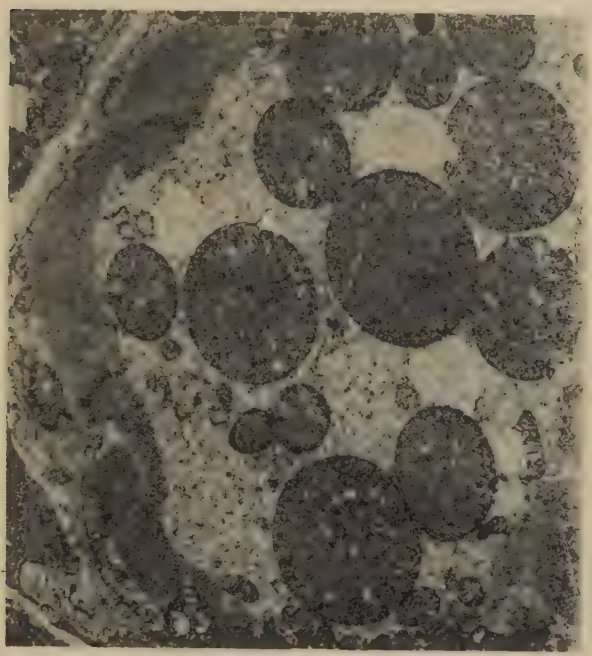
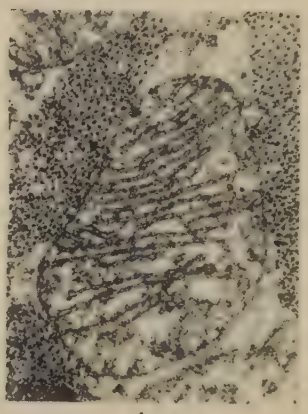


Fig. 15.3c 1 µm



1 µm

Fig. 15.3d

between the longitudinal and the transverse system is seen in Fig. 15.5.a and 15.5.b and it can either be diadic or triadic (between one or two elements of the longitudinal system (LS) with one element of the transverse system (TS)).

Discussion

The greater number of mitochondria per brown adipocyte in hedgehog than in non-hibernating summer hedgehog is quite in accordance with the thermogenesis produced during arousals. Kristoffersson and Soivio (1964) have shown that hedgehogs arouse once every 1-2 weeks during the hibernation. Brown adipose tissue has a high capacity for thermogenesis (Ball 1965, Smith and Hock 1963), which is achieved through a very high oxidative metabolism (for review see Lindberg 1970). This high oxidative metabolism is reflected in the high activity of malic dehydrogenase (MDH) (Chapter 11). It is also interesting that the concentration of soluble proteins in brown fat is higher during hibernation than during the other seasons (Chapter 11). Paleus and Johansson (1968) found higher values of lipid-free dry weight in brown fat from hibernating than from non-hibernating hedgehog. As the sarcoplasmic membranes are almost absent in brown adipocytes (Afzelius 1970) and no remarkable seasonal changes in the size of the nucleus have been found, it is possible to correlate the number of mitochondria with the concentration of cytochrome C (Paleus and Johansson 1968), lipid-free dry weight and soluble proteins (Chapter 11). Umahara (1968) did also find seasonal changes in the number of mitochondria; a higher population density of mitochondria in brown adipocytes from the bat (Rhinolophus ferrum-equinum nippon) in October than in April. The size of mitochondria from brown adipocytes of hedgehog and the arrangement of tightly packed cristae, which are straight or gently

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Fig. 15.4.a. Heart muscle cells from hibernating hedgehog (II) seen in the direction of the muscle fibers (FI). Two parts of the intercalated disc are seen desmosomes (D) and nexus (NE). The continuity between the inercalated disc and the sarcolemma (S) is seen. Magnification 19 000 x.

Fig. 15.4.b. Heart muscle cells from hibernating hedgehog (II) seen in a direction perpendicular to the muscle fibers (FI). Regions of myofibrillar insertion (MI) and nexus (NE) along an intercalated disc and the lateral system (LS) of the sarcoplasmic reticulum are seen. Magnification 19 000 x.

Fig. 15.5.a. Heart muscle cell from hibernating hedgehog (II) showing the bands of the myofibers (FI), the lateral system (LS) and the transvera system (TS) of the sarcoplasmic reticulum and the coupling between LS and TS. Magnification 32 000 x.

Fig. 15.5.b. Heart muscle cell from guinea-pig showing the same as Fig. 15.5.a. Magnification 9 600 x.

The line below the micrographs represents 1 µm.

Fig. 15.4a

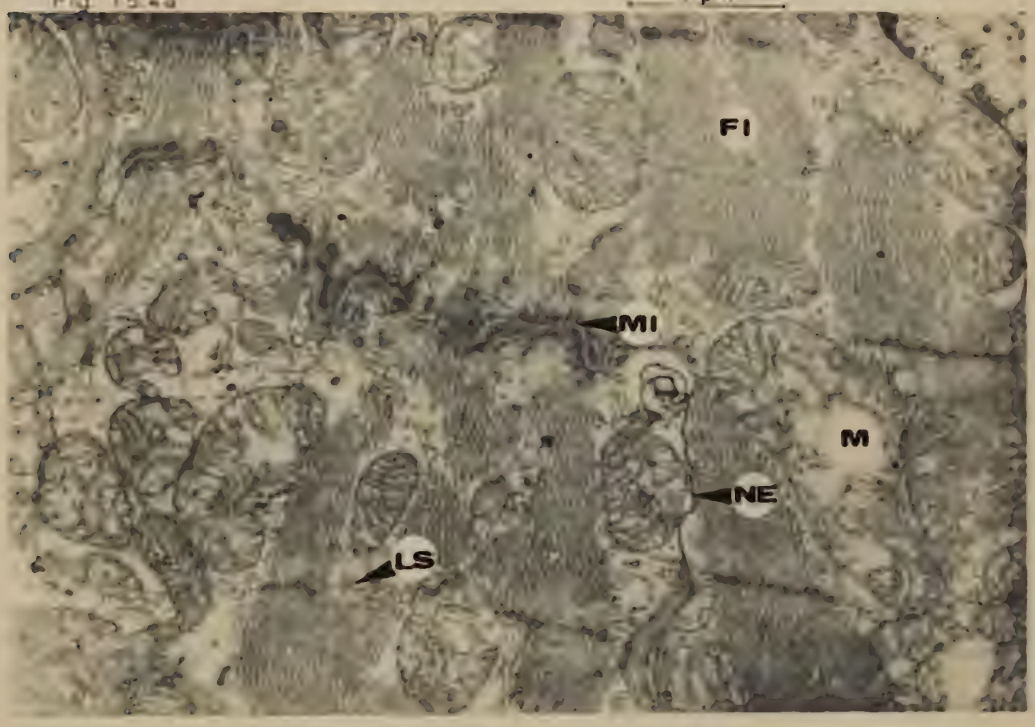

Fig. 15.4b

Fig. 15.5a

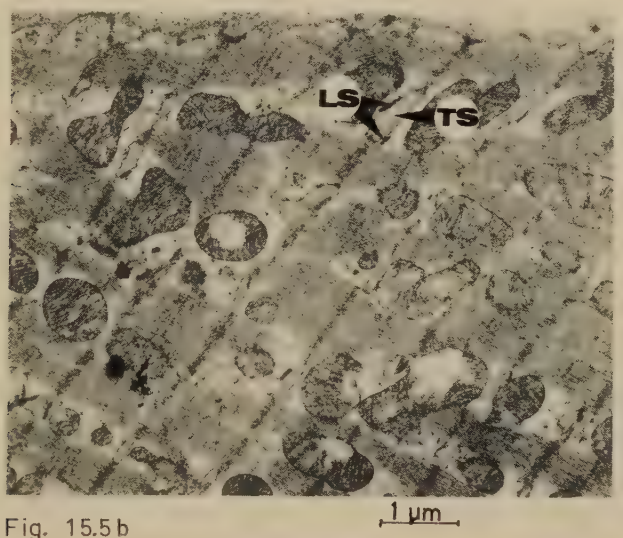

Fig. 15.5b

curved and transverse the whole width of the mitochondria is quite in agreement with those of other mammals (Afzelius 1970). The multilocularity, which is one of the characteristics of brown adipocytes is most typical during the hibernation of hedgehog. The lipid droplets increase in size from 1-5 μ to 5-10 μ from winter to summer. The small size of fat droplets during hibernation favors the convenience of access of fat for catabolism. This implies that the lipid content per adipocyte is greater during summer than during hibernation, which is supported by the work of Paleus and Johansson (1968), who found maximum concentration of lipids in hedgehog brown fat during May-September. The same results have been found by Suomalainen and Herlevi (1950), who found the most intensely sudan black stained brown adipocytes from summer hedgehogs, and by Zirm (1956), who found a higher lipid content in brown fat from non-hibernating hedgehog than from hibernating. It is obvious from these results that the synthesis of lipids starts almost immediately after the arousal of hedgehog, as has been pointed out by Boerner-Patzelt (1958). This is also supported by the increase in activity of glucose-6-phosphate dehydrogenase (G-6-PDH) between winter (II) and spring (III) in brown fat of hedgehog (Chapter 11). In the bat (Rhinolophus ferrum-equinum nippon), however, Umahara (1968) revealed a strong depletion of fat droplets from brown adipocytes in April and a conspicuous atrophy of brown adipocytes in August. This change was correlated to a possible resting state in summer. It is evident that there are great species differences in the seasonal changes of brown adipocytes, and that it is impossible to draw any conclusions from the results from one specie to another.

The brown adipose tissue of hedgehogs from the spring season (III) (see Fig. 2.1.) has the same ultrastructural appearance as from the hibernating season (II) (Fig. 15.6).

This is quite in agreement with the fact that these animals were almost hibernating (Chapter 2). Consequently the brown adipose tissue from these spring hedgehogs is neither disintegrated nor depleted of fat (see Paleus and Johansson 1968) after all the arousals during the hibernation, which implies that these animals have some reserve capacity.

Afzelius (1970) has reviewed the anatomy and cytology of brown adipose tissue and characterized the plasma membrane by its numerous invaginations. Hedgehog brown adipose tissue has not been well investigated with regard to these invaginations which are generally considered to be an expression of pinocytotic activity.

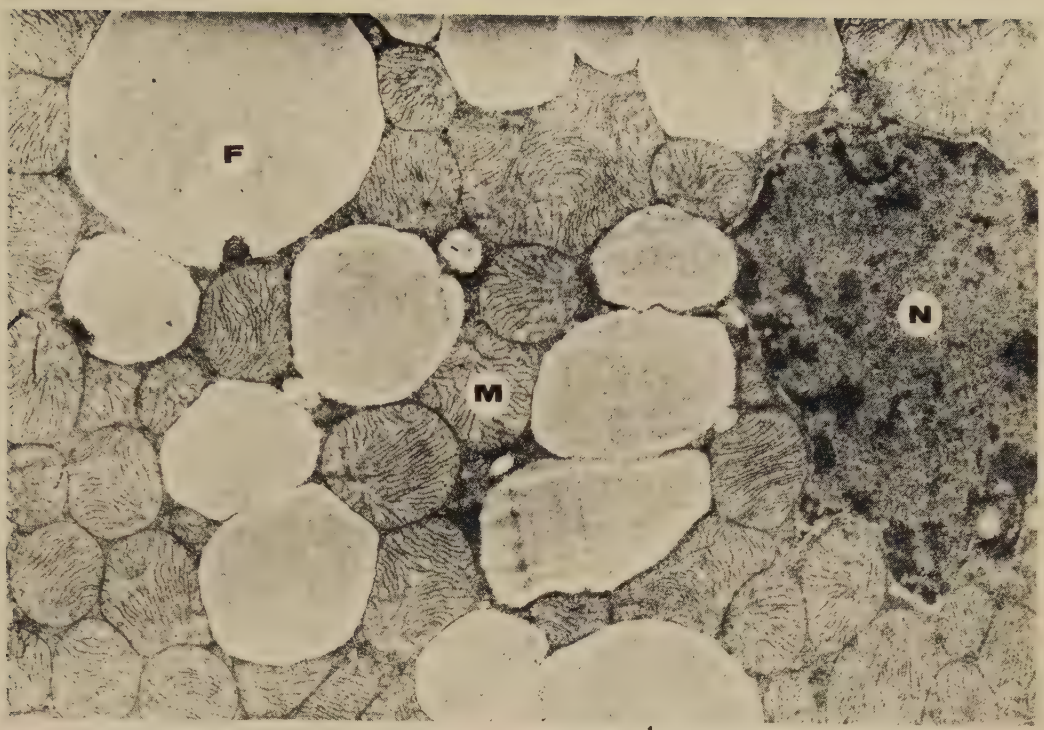

Fig. 15.6a

Fig. 15.6b

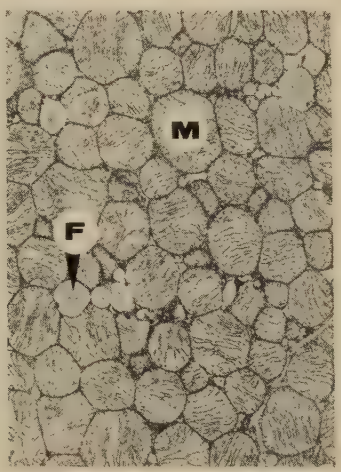

Fig. 15.6c

No differences in the structure of myocardium from guinea-pig, hibernating or non-hibernating hedgehog were observed. The absence of seasonal changes of the number of mitochondria in myocardium is supported by the result that mitochondrial-MDH does not show any difference in percental activity from hibernating or non-hibernating hedgehog (Chapter 11). Poche (1959) found in the myocardium of hibernating fat dormouse (Glis glis) that the number of mitochondria was constant. The number of cristae and the contrast of the mitochondria increased however, in comparison with the non-hibernatory state. On the other hand Moreland (1962) found an increase in the number and size of mitochondria from the myocardium of the ground squirrel (Citellus tridecemlineatus) when hibernating, compared to non-hibernating. Zimny and Moreland (1968) confirmed these results on the same specie. During arousals the number of mitochondria decreased and the succinic dehydrogenase activity increased. Zimny and Moreland (1968) proposed that the increase of the number of mitochondria depends on increased fatty acid oxidation during hibernation, which is supported by the proposal that the turnover of lipids in myocardium is increased during the hibernation of hedgehog (Chapter 11). The low value of the activity of succinic dehydrogenase during hibernation of ground squirrel (Citellus tridecemlineatus) may be an expression of low aerobic metabolism in the myocardium of hibernating animals. This is supported by the high anaerobic capacity of hibernators (Chapter 11). Rosenquist and Zimny (1969) observed differences in the myocardial ultrastructure between hibernating and non-hibernating ground squirrels (Citellus tridecemlineatus). The sarcoplasmic reticulum was more abundant during hibernation, and the terminal cisternae or lateral sacs of the myocardial triad were more electron dense than those not hibernating. In addition, the transverse tubules near the cell periphery were greater in diameter in the myocardium of hibernating ground squirrels. They also observed that the cell coat thickness was thick (350 Å) during hibernation but only 250 Å in the non-hibernating state. These results were later confirmed by Rosenquist (1970), who suggested that these ultrastructural changes may enhance calcium uptake and thereby facilitate excitation - contraction coupling during hibernation.

Fig. 15.6.a. A typical brown adipocyte from spring hedgehog (III). It is the same appearance as from hibernating hedgehog. Magnification 9 600 x.

Fig. 15.6.b. A part of a brown adipocyte from spring hedgehog (III) with many fat droplets (F). Magnification 6 000 x.

Fig. 15.6.c. A part of a brown adipocyte from spring hedgehog (III) with very few and small fat droplets (F). Magnification 6 000 x.

The line below the micrographs represents 1 µm.

Zimny et al. (1968) observed that the intermembrane cleft in the intercalated discs were widened at 15° C-body temperature of rats, and that the width of the intermembrane cleft was maintained and that the intracytoplasmatic density was increased in ground squirrels (Citellus tridecemlineatus) at body temperatures of 5° C compared to normal animals. This effect in rats is difficult to interpret in physiological terms, as Barr et al. (1965) have shown that the desmosomal junction is a path of low resistance for the action potentials. The action and the membrane potentials of rabbit atria have been shown to diminish rapidly between 25-15° C, but this is not the case for ground squirrels (Citellus tridecemlineatus) between 31-6° C body temperature (Marshall and Willis 1962). Consequently there are also great differences in the myocardial membranes from hibernators and non-hibernators besides the biochemical differences (Chapter 11).

The changes of the myocardial ultrastructure reported by Rosenquist and Zimny (1969) and Rosenquist (1970) in the myocardium of the ground squirrel (Citellus tridecemlineatus) can neither be supported by this investigation on hedgehogs nor by that of Aloia and Pengelley (1971) on Citellus lateralis, which is a close relative to Citellus tridecemlineatus. Aloia and Pengelley (1971) did not find any ultrastructural differences in the myocardium of hibernating and non-hibernating ground squirrels or between the ground squirrel and other mammalian species, which is quite in accordance to the results of this study on hedgehogs and guinea-pigs.

ADROUNY, G.A., Differential patterns of glycogen metabolism in cardiac and skeletal muscles. Amer. J. Physiol. 1969. 217. 686.

AFZELIUS, B.A., Brown adipose tissue: It's gross anatomy, histology, and cytology. In Brown Adipose Tissue (Edited by Lindberg, O.) Elsevier, 1970.

AGID, R. and L. AMBID, Effects of corporeal temperature on glucose metabolism in a homeotherm, the rat, and a hibernator, the garden dormouse. In Depressed Metabolism (Edited by Musacchia, X.J. and J.F. Saunders) Elsevier, 1969. 119.

ALOIA, R.C. and E.T. PENGELLEY, Ultrastructure of the ventricular tissue of the hibernating ground squirrel, Citellus lateralis, in relation to the physiology of the hibernator's heart. Comp. Biochem. Physiol. 1971. 38. 517.

ANDJUS, R.K., T. CIRKOVIC, N. CUPERLOVIC, J. DAVIDOVIC, V. MAKOVIC-USKOKOVIC and T. VELIMIROVIC, Brain metabolism and resistance of a hibernator (Citellus citellus) and the rat to different anoxic conditions, including cardiac arrest in deep hypothermia. Ann. Acad. Sci. Fenn., Ser. A, IV. 1964. 71. 11.

BALL, E.G., Some energy relationships in adipose tissue. Ann. N.Y. Acad. Sci. 1965. 131. 225.

BARR, L., M.M. DEWEY and W. BERGER, Propagation of action potentials and the structure of the nexus in cardiac muscle. J. gen. Physiol. 1965. 48. 797.

BAUMBER, J. and A. DENYES, Acetate-1-C^{14} metabolism of white fat from hamsters in cold exposure and hibernation. Amer. J. Physiol. 1963. 205. 905.

BIÖRCK, G. and B.W. JOHANSSON, Comparative studies on temperature effects upon the electrocardiogram in some vertebrates. Acta physiol. scand. 1955. 34. 257.

BIÖRCK, G., B.W. JOHANSSON and H. SCHMID, Reactions of hedgehogs, hibernating and non-hibernating, to the inhalation of oxygen carbon dioxide and nitrogen. Acta physiol. scand. 1956a. 37. 71.

BLATT, W.F., J. WALKER and M. MAGER, Tissue lactic dehydrogenase isozymes: Variation in rats during prolonged cold exposure. Amer. J. Physiol. 1965. 209. 785.

BOERNER-PATZELT, D., Das braune Fett der sogenannten Winterschlafdrüse des Igels. Z. mikr.-anat. Forsch. 1958. 63. 5.

BOYER, S.H., D.C. FAINER and E.J. WATSON-WILLIAMS, Lactate dehydrogenase variant from human blood: Evidence for molecular subunits. Science (N.Y.) 1963. 141. 642.

BURLINGTON, R.F. and J.E. WIEBERS, Anaerobic glycolysis in cardiac tissue from a hibernator and a non-hibernator as effected by temperature and hypoxia. Comp. Biochem. Physiol. 1966. 17. 183.

BURLINGTON, R.F., Gluconeogenesis in kidney cortex slices from cold-exposed rats and hamsters. Comp. Biochem. Physiol. 1966. 17. 1049.

BURLINGTON, R.F. and G.J. KLAIN, Effect of hypoxia on gluconeogenesis in the albino rat and thirteen-lined ground squirrel (Citellus tridecemlineatus). Comp. Biochem. Physiol. 1967. 20. 275.

BURLINGTON, R.F. and J.H. SAMPSON, Distribution and activity of lactic dehydrogenase isozymes in tissues from a hibernator and a non-hibernator. Comp. Biochem. Physiol. 1968. 25. 185.

BÜCHER, T. and M. KLINGENBERG, Wege des Wasserstoffs in der lebendigen Organization. Angew. Chem. 1958. 70. 552.

CAHN, R.D., N.O. KAPLAN, L. LEVINE and E. ZWILLING, Nature and development of lactic dehydrogenases. Science (Lond) 1962. 136. 962.

CAHN, R.D., Cellular damage and the control of lactic dehydrogenase synthesis in cell cultures by oxidative metabolites. J. Cell. Biol. 1963. 19. (2.) 12A.

CAHN, R.D., Developmental changes in embryonic enzyme patterns: the effect of oxidative substrates on lactic dehydrogenase in beating chick embryonic heart cell cultures. Dev. Biol. 1964. 9. 327.

CHAFFEE, R.R.J., F.L. HOCH and C.P. LYMAN, Mitochondrial oxidative enzymes and phosphorylations in cold exposure and hibernation. Amer. J. Physiol. 1961. 201. 29.

CHAFFEE, R.R.J., J. ALLEN, Y. CASSUTO and R. SMITH, Biochemistry of brown fat and liver in cold acclimatized hamsters. Amer. J. Physiol. 1964. 207. 1211.

CHAFFEE, R.R.J., E.T. PENGELLEY, J.R. ALLEN and R.E. SMITH, Biochemistry of brown fat and liver of hibernating golden-mantled ground squirrels (Citellus lateralis). Canad. J. Physiol. Pharmacol. 1966. 44. 217.

COVINO, B.G. and J.P. HANNON, Myocardial metabolic and electrical properties of rabbits and ground squirrels at low temperatures. Amer. J. Physiol. 1959. 197. 494.

DAWSON, D.M., T.L. GOODFRIEND and N.O. KAPLAN, Lactic dehydrogenases: functions of the two types rates of synthesis of the two major forms can be correlated with metabolic differentiation. Science (N.Y.) 1964. 143. 929.

DENYES, A. and J. HASSETT, A study of the metabolism of liver, diaphragm and kidney in cold-exposed and hibernating hamsters. In Mammalian Hibernation (Edited by Lyman, C.P. and A.R. Dawe) Bull. Mus. Comp. Zool. (Harv) 1960. 124. 437.

DENYES, A. and J.D. CARTER, Utilization of acetate-1-C^{14} by hepatic tissue from cold-exposed and hibernating hamsters. Amer. J. Physiol. 1961. 200. 1043.

DEWEY, M.M., The structure and function of the intercalated disc in vertebrate cardiac muscle. In Comparative Physiology of the heart: Current trends. (Edited by McCann, F.V.) Basel-Stuttgart. 1969. Experientia Supplementum 15.

DRASKOCZY, P.R. and C.P. LYMAN, Turnover of catecholamines during hibernation. Pharmacologist 1965. 7. 167.

DRYER, R.L. and J.R. PAULSRYD, Effect of arousal on ATP levels in bats. Fed. Proc. 1966. 25. (4) 1293.

EDWARDS, B.A. and K.A. MUNDAY, The function of brown fat in the hedgehog (Erinaceus europaeus). Comp. Biochem. Physiol. 1969a. 30. 1029.

ESTABROOK, R.W. and B. SACKTOR, α-Glycerophosphate oxidase of flight muscle mitochondria. J. Biol. Chem. 1958. 233. 1014.

FEIST, D.D. and W.B. QUAY, Effects of cold acclimation and arousal from hibernation on brown fat lipid and protein in the golden hamster (Mesocricetus auratus). Comp. Biochem. Physiol. 1969. 31. 111.

FINE, J.H., N.O. KAPLAN and D. KUFTINEC, Developmental changes of mammalian lactic dehydrogenases. Biochemistry 1963. 2. 116.

FLATT, J.P. and E.G. BALL, Studies on the metabolism of adipose tissue - XV. An evaluation of the major pathways of glucose catabolism as influenced by insulin and epinephrine. J. Biol. Chem. 1964. 239. 675.

FORSBERG, A. and H.S.S. SARAJAS, Studies on the metabolism of ^{14}C-labelled glucose in awake and hibernating hedgehogs. Ann. Acad. Sci. Fenn. Ser. A, IV. 1955. 28. 3.

FREHN, J.L., Mitochondrial respiration in mammalian liver during cold exposure and hibernation. Trans. Ill. St. Acad. Sci. 1966. 59. 342.

GALSTER, W.A. and P. MORRISON, Cyclic changes in carbohydrate concentrations during hibernation in the arctic ground squirrel. Amer. J. Physiol. 1970. 218. 1228.

GOODFRIEND, T.L. and N.O. KAPLAN, Induction of changes in the subunit composition of lactic dehydrogenase. J. Cell. Biol. 1963. 19. (2.) 28A.

GREEN, D.E., α-Glycerophosphate dehydrogenase. Biochem. J. 1936. 30. 629.

HANNON, J.P., D.A. VAUGHAN and R. HOCK, Endogenous tissue respiration of the arctic ground squirrel as affected by hibernation and season. J. Cell. Comp. Physiol. 1961. 57. 5.

HANNON, J.P. and D.A. VAUGHAN, Initial stages of intermediary glucose catabolism in the hibernator and non-hibernator. Amer. J. Physiol. 1961. 201. 217.

HANSON, A. and B.W. JOHANSSON, Myocardial lactate concentration in guinea-pigs, normothermic and hypothermic, and hedgehogs, in a hibernating and a non-hibernating state. Acta physiol. scand. 1961. 53. 137.

HARDMAN, M.J. and D. HULL, Fat metabolism in brown adipose tissue in vivo. J. Physiol. 1970. 206. 263.

HAWLEY, P.L., Energy sources in hibernator heart: pyridine nucleotide metabolism. Proc. Soc. exp. Biol. (N.Y.) 1968. 129. 430.

HENDE, van den C., E. MUYLLE and W. OYAERT, Enzyme activities of liver, heart- and skeletal muscles of the pig. Zbl. Vet.-Med. A. 1968. 15. 135.

HIESTRAND, W.A., W.T. ROCKHOLD, F.W. STEMLER, D.E. STULLKEN and J.E. WIEBERS, The comparative hypoxic resistance of hibernators and non-hibernators. Physiol. Zool. 1950. 23. 264.

HORWITZ, B.A. and L. NELSON, Effect of temperature on mitochondrial respiration in a hibernator (Myotis austroriparius) and a non-hibernator (Rattus rattus). Comp. Biochem. Physiol. 1968. 24. 385.

JOEL, C.D., The physiological role of brown adipose tissue. In Handbook of Physiology, Sec 5: Adipose tissue (Edited by Renold, A.E. and G.F. Cahill, Jr.) 1965. 59.

JOHANSSON, B.W., Brown fat: A review. Metabolism 1959. 8. 221.

JOHANSSON, B.W., Heart and circulation in hibernators. In Mammalian Hibernation III. (Edited by Fisher, K.C. et al.) American Elsevier, New York. 1967. 200.

JOHANSSON, B.W., Electrocardiographic changes in depressed metabolism. In Depressed Metabolism. (Edited by Musacchia, X.J. and J.F. Saunders) Elsevier. 1969a. 313.

JOHANSSON, B.W., Temperature dependence of cholinesterase and lactate dehydrogenase in the guinea-pig, hedgehog and codfish. Acta physiol. scand. 1969b. 77. 1.

KARLSSON, B.W. and E.J. CARLSSON, Levels of lactic and malic dehydrogenase isoenzymes in mammary gland, milk and blood serum of the rat during pregnancy, lactation and involution. Comp. Biochem. Physiol. 1968. 25. 949.

KARLSSON, B.W. and L.S. PALMER, Lactic dehydrogenase isozyme distribution in various tissue fractions of the developing mammalian liver. Comp. Biochem. Physiol. 1971. 38B. 299.

KAPLAN, N., Thermodynamics and mechanism of the phosphate bond. In The Enzymes. New Yor 1951. II. 55.

KAPLAN, N.O. and R.D. CAHN, Lactic dehydrogenases and muscular dystrophy in the chicken. Proc. nat. Acad. Sci. (Wash.) 1962. 48. 2123.

KAPLAN, N.O., Symposium on multiple forms of enzymes and control mechanisms. I. Multiple forms of enzymes. Bact. Rev. 1963. 27. 155.

KAPLAN, N.O., Lactate dehydrogenase-structure and function. Brookhaven Symp. Biol. 1964. 17. 131.

KAYSER, C., The Physiology of Natural Hibernation. Pergamon Press, Oxford. 1961.

KAYSER, C. and A. MALAN, Central nervous system in hibernation. Experientia 1963. 19. 441.

KITTO, G.B. and A.C. WILSON, Evolution of malate dehydrogenase in birds. Science (N.Y.) 1966. 153. 1408.

KONTTINEN, A., M. RAJASALMI and H.S.S. SARAJAS, Fat metabolism of the hedgehog during the hibernating cycle. Amer. J. Physiol. 1964. 207. 845.

KORNACKER, M.S. and E.G. BALL, Citrate cleavage in adipose tissue. Proc. nat. Acad. Sci. (Wash.) 1965. 54. 899.

KRISTOFFERSSON, R. and A. SOIVIO, Hibernation in the hedgehog (Erinaceus europaeus L.). Ann. Acad. Sci. Fenn. Ser. A IV. 1964. 82. 3.

KRISTOFFERSSON, R., A. SOIVIO and P. SUOMALAINEN, Studies on the physiology of the hibernating hedgehog. Ann. Acad. Sci. Fenn. Ser. A IV. 1965. 92. 3.

LATNER, A.L. and A.W. SKILLEN, Isoenzymes in biology and medicine. Academic Press, London. 1968.

LAYNE, E., In Methods in Enzymology. (Edited by Colowick, S.P. and N.O. Kaplan). Acad. Press, New York. 1957. Vol III, Section IV. 451.

LINDBERG, O., ed., Brown Adipose Tissue. Elsevier. 1970.

LINDSAY, D.T., Isozymic patterns and properties of lactate dehydrogenase from developing tissues of the chicken. J. exp. Zool. 1963. 152. 75.

LINDY, S. and M. RAJASALMI, Lactate dehydrogenase isozymes of chick embryo: response to variations of ambient oxygen tension. Science (N.Y.) 1966. 153. 1401.

LIU, C.-C., J.L. FREHN and A.D. LaPORTA, Liver and brown fat mitochondrial response to cold in hibernators and non-hibernators. J. appl. Physiol. 1969. 27. 83.

LOWENSTEIN, J.M., The pathway of hydrogen in biosynthesis - I. Experiments with glucose-1-H^3 and lactate-2-H^3. J. Biol. Chem. 1961. 236. 1213.

MAGER, M., W.F. BLATT and R.W. NEWMAN, Lactic dehydrogenase isozymes: variations in the plasma of men exposed to cold. J. appl. Physiol. 1968. 24. 616.

MARKERT, C.L., Lactate dehydrogenase isozymes: dissociation and recombination of subunits. Science (N.Y.) 1963. 140. 1329.

MARSHALL, J.M. and J.S. WILLIS, The effects of temperature on the membrane potentials in isolated atria of the ground squirrel, Citellus tridecemlineatus. J. Physiol. (Lond.) 1962. 164. 64.

MICHAEL, C.R. and M. MENAKER, The effect of temperature on the isolated heart of the bat, Myotus lucifugus. J. cell. comp. Physiol. 1963. 62. 355.

MILLONIG, G., Advantages of a phosphate buffer for OsO_4 solutions in fixation. J. appl. Physiol. 1961. 32. 1637.

MOKRASCH, L.C., H.J. GRADY and S. GRISOLIA, Thermogenic and adaptive mechanisms in hibernation and arousal from hibernation. Amer. J. Physiol. 1960. 199. 945.

MORELAND, J.E., Electronmicroscope studies of mitochondria in cardiac and skeletal muscle from hibernated ground squirrels. Anat. Rec. 1962. 142. 155.

OLSSON, S.-O. R., Unpublished results 1971.

PALEUS, S. and B.W. JOHANSSON, Seasonal variations in cytochrome c content of hedgehog brown adipose tissue. Acta chem. scand. 1968. 22. 342.

PALMER, L.S. and B.W. KARLSSON, Lactic dehydrogenase isozyme distributions in various cell types of the mammalian liver. Canad. J. Biochem. 1969. 47. 1171.

PANDE, S.V., R. PARVIN KHAN and T.A. VENKITASUBRAMANIAN, Nicotinamide adenine dinucleotide phosphate-specific dehydrogenases in relation to lipogenesis. Biochim. biophys. Acta 1964. 84. 239.

PAULSRUD, J.R., K.G. MANN and R.L. DRYER, A comparison of rat and bat malic dehydrogenase isoenzymes. In Brown Adipose Tissue. (Edited by Lindberg, O.) Elsevier, New York. 1970. Chap. 8. 197.

PLAGEMANN, P.G.W., K.F. GREGORY and F. WROBLEWSKI, Die electrophoretisch trennbaren Lactat-dehydrogenasen des Säugetieres. III. Einfluss der Temperatur auf die Lactat-dehydrogenasen des Kaninchens. Biochem. Z. 1961. 334. 37.

POCHE, R., Elektronenmikroskopische Untersuchungen zur Morphologie des Herzmuskels vom Siebenschläfer während des aktiven und des lethargischen Zustandes. Z. Zellforsch. 1959. 50. 332.

POPOVIC, V.P., Cardiac output in hibernating ground squirrels. Amer. J. Physiol. 1964. 207. 1345.

PRECHT, H., J. CHRISTOPHERSEN and H. HENSEL, Temperatur und Leben. Springer-Verlag, Berlin. 1955.

PRUSINER, S., J.R. WILLIAMSON, B. CHANCE and B.M. PADDLE, Pyridine nucleotide changes during thermogenesis in brown fat tissue in vivo. Arch. Biochem. 1968. 123. 368.

PRUSINER, S., B. CANNON and O. LINDBERG, Mechanisms controlling oxidative metabolism in brown adipose tissue. In Brown Adipose Tissue. (Edited by Lindberg, O.) Elsevier, New York. 1970. Chap. 13. 283.

RASMUSSEN, A.T., The so-called hibernating gland. J. Morph. 1923. 38. 147.

RATHS, P., Ueber das Serum-Natrum-Kalium und Kalzium des winterschlafenden und hypothermischen Hamsters. Z. Biol. 1962. 113. 173.

REBEL, G., J.D. WEILL, P. MANDEL and C. KAYSER, Y a-t-il formation de glycogène à partir des acides gras chez les hibernants en sommeil hivernal? C.R. Soc. Biol. (Paris) 1960. 154. 2118.

ROGNSTAD, R. and J. KATZ, Gluconeogenesis in the kidney cortex. Effects of D-malate and amino-oxyacetate. Biochem. J. 1970. 116. 483.

ROSENQVIST, T.H. and M.L. ZIMNY, Modification of the cell surface and the muscular triad in the heart of a hibernator. The Physiologist 1969. 12. 343.

ROSENQVIST, T.H., Ultrastructural changes in the plasma membrane and sarcoplasmic reticulum of myocardial cells during hibernation. Cryobiology 1970. 7. 14.

SACKTOR, B., A biochemical basis of flight muscle activity. Proc. IVth. int. Con. Biochem. 1959. 12. 138.

SALTHE, S.N., Comparative catalytic studies of lactic dehydrogenases in the amphibia: environmental and physiological correlations. Comp. Biochem. Physiol. 1965. 16. 393.

SENTURIA, J.B., S. STEWART and M. MENAKER, Rate-temperature relationships in the isolated hearts of ground squirrels. Comp. Biochem. Physiol. 1970. 33. 43.

SIMMONDS, R.C., E.C. LARKIN and F. ULVEDAL, Lactic dehydrogenase activity in hibernating and active mexican ground squirrels (Spermophilus mexicanus) exposed to hypertoxic and normoxic atmosphere. Hibernation-Hypothermia IV Symposium 1971. 4. (Abstr.)

SMALLEY, R.C. and R.L. DRYER, Brown fat in hibernation. In Mammalian Hibernation III. (Edited by Fisher, K.C. et al.) American Elsevier, New York. 1967. 325.

SMITH, R.E. and R.J. HOCK, Brown fat: thermogenic effector of arousal in hibernators. Science 1963. 140. 199.

SMITH, R.E., Thermogenesis and thyroid action. Nature 1964. 204. 1311.

SOUTH, F.E., Rates of oxygen consumption and glycolysis of ventricle and brain slices obtained from hibernating and non-hibernating mammals, as a function of temperature. Physiol. Zool. 1958. 31. 6.

SOUTH, F.E., Hibernation, temperature and rates of oxidative phosphorylation by heart mitochondria. Amer. J. Physiol. 1960a. 198. 463.

SOUTH, F.E., Some metabolic specializations in tissues of hibernating mammals. In Mammalian Hibernation. (Edited by Lyman, C.P. and A.R. Dawe) Bull. Mus. Comp. Zool. (Harv.) 1960b. 124. 475.

SOUTH, F.E. and W.A. HOUSE, Energy metabolism in hibernation. In Mammalian Hibernation III. (Edited by Fisher, K.C. et al.) American Elsevier, New York. 1967. 305.

SUOMALAINEN, P. and A.-M. HERLEVI, The hibernating gland and the alarm reaction. Arch. Soc. Zool. Botan. Fenn. 'Vanamo' 1950. 5:2. 72.

TASHIMA, L.S., S.J. ADELSTEIN and C.P. LYMAN, Radioglucose utilization by active, hibernating, and arousing ground squirrels. Amer. J. Physiol. 1970. 218. 303.

TEPPERMANN, H.M. and J. TEPPERMANN, Hexosemonophosphate shunt and adaptive lipogenesis. Diabetes 1958. 7. 478.

TEPPERMANN, J. and H.M. TEPPERMANN, Metabolism of glucose-1-C^{14} and glucose-6-C^{14} by liver slices of refed rats. Amer. J. Physiol. 1961. 200. 1069.

TWENTE, J.A. and J.W. TWENTE, Concentrations of ℓ-lactate in the tissues of Citellus lateralis after known intervals of hibernating periods. J. Mammal. 1968. 49. 541.

UMAHARA, Y., Light and electron microscopic studies on the brown adipose tissue in the bat. Arch. Histol. Jap. 1968. 29. 459.

UTTER, M.F. and K. KURAHASHI, Mechanism of action of oxalacetate carboxylase. J. biol. Chem. 1954. 207. 821.

WEINER, J.D. and E.P. STEYN-PARVÉ, The metabolism of glucose in normal and thiamine-deficient pigeons. I. The conversion of glucose into glycogen, fatty acids and respiratory carbon dioxide. Biochim. biophys. Acta 1959. 35. 473.

WHITTEN, B.K. and G.J. KLAIN, Protein metabolism in hepatic tissue of hibernating and arousing ground squirrels. Amer. J. Physiol. 1968a. 214. 1360.

WHITTEN, B.K. and G.J. KLAIN, NADP specific dehydrogenases and hepatic lipogenesis in the hibernator. Comp. Biochem. Physiol. 1968b. 29. 1099.

WHITTEN, B.K., Regulation of hepatic protein synthesis in the hibernator. Hibernation-Hypothermia IV Symposium. Snowmass, Aspen, Colorado. 1971.

WILKINSON, J.H., Isoenzymes. E. and F.N. Spon Ltd., London. 1965.

WILSON, A.C., R.D. CAHN and N.O. KAPLAN, Functions of the two forms of lactic dehydrogenase in the breast muscle of birds. Nature (Lond.) 1963. 197. 331.

ZIMNY, M.L., Metabolism of some carbohydrate and phosphate compounds during hibernation in the ground squirrel. J. cell. comp. Physiol. 1956. 48. 371.

ZIMNY, M.L. and J.E. MORELAND, Mitochondrial populations and succinic dehydrogenase in the heart of a hibernator. Canad. J. Physiol. Pharmacol. 1968. 46. 911.

ZIMNY, M.L., M. SHERMAN and C. ROMANO, Ultrastructural modifications of the intercalated disc during hypothermia in the rat and the ground squirrel. Cryobiology 1968. 4. 317.

ZIRM, K.L., Ein Beitrag zur Kenntnis des natürlichen Winterschlafes und seines regulierenden Wirkstoffes I. Z. Naturforsch. 1956. 11b. 530.

Comparative studies on the temperature dependence of Lactic- and Malic dehydrogenase from a homeotherm, guinea pig (Cavia porcellus), two hibernators, hedgehog (Erinaceus europaeus) and bat (Nyctalus noctula); and two poikilotherms, frog (Rana temporaria) and cod (Gadus callarias).

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Abstract

1. The temperature effects on V_{max} and the isoenzyme distribution of LDH and MDH from tissue homogenates from guinea pig (Cavia porcellus), hedgehog (Erinaceus europaeus) (both hibernating and non-hibernating) bat (Nyctalus noctula) (hibernating) and frog (Rana temporaria) and cod (Gadus callarias) were examined.

2. Correlations were found between the LDH-H activity and the energy of activation and inactivation of LDH.

3. No correlation was found between the energy of activation of MDH and the MDH-isoenzyme distribution.

4. No differences in the energy of activation of LDH or MDH were found between the animals.

5. However, MDH from the mammals had higher temperature maxima than MDH from the poikilotherms.

Introduction

Different cold resistance of poikilotherms, hibernators and homeotherms have been shown both *in vivo* and *in vitro* (Adolph 1951, Smith 1957, Kayser 1957, Covino and Hannon 1959, Lyman and Blinks 1959, Dawe and Landau 1960, Mokrash *et al.*, 1960, Zimny and Taylor 1965, Burlington and Wiebers 1967, Johansson 1967 and 1969a, Bowler and Duncan 1968 a and b, 1969, Senturia *et al.*, 1970). The critical temperature at which heart beat of homeotherms ceases is between 10-20°C, depending on the species and the cooling rate.

McMurchie *et al.*, (1973), correlated the change in heart rate at 21°C for the homeotherm and rabbit, with both temperature induced phase changes in the lipid components of membranes, and the change in the activation energy of membrane associated ATPases. None of these changes were observed in the membranes of the poikilothermic heart (*Bufo marinus*). In the same way, Lenaz *et al.*, (1972), Lyons and Raison (1970) and Raison *et al.*, (1971 a and b) have suggested that the difference in temperature resistance between hibernators, poikilotherms and homeotherms lies in the temperature response of the membranes (Chapman 1969, Somero 1972).

It has also been discussed whether differences in E_a (the energy of activation) of the metabolism of specific and critical enzymes could explain the difference between hibernators, poikilotherms and homeotherms (Vroman and Brown 1963, Baldwin and Hochachka 1970, Hochachka and Somero 1971, Hannon *et al.*, 1972). South (1958, 1960 a and b) has found differences in E_a between homeotherms and hibernators and between hibernating and non-hibernating hibernators. The E_a values were highest for homeotherms and lowest for hibernating animals.

Roberts and Chaffee (1973) found that activation energy of succinoxidase from heart (42-46 kJ/mole) did not markedly differ between cold

acclimated, hibernating or control hamsters (Mesocricetus auratus), cold acclimated and control rats and control squirrel monkeys (Saimiri sciureus) at the temperature range 15-37°C, but at 6-15°C the energy of activation (47 kJ/mole) was much higher for squirrel monkeys. The Arrhenius plots of the hearts from the other animals showed a break at 15°C with a lower energy of activation below 15°C (5.8-27.6 kJ/mole). This may depend on a decrease of K_m (Michaelis constant) below 15°C in the rat and hamster but not in the squirrel monkey. The values for E_a of cardiac succinoxidase (15-37°C) found by Roberts and Chaffee (1973) are supported by Hannon (1958) (rat), and Covino and Hannon (1959) (rabbit and ground squirrel). An interesting fact is that South (1960) and Covino and Hannon (1959) found higher values for the oxidation of pyruvate + malate by sarcosomes from rat and hamster hearts. The E_a values for cardiac succinoxidase from rat and hamster are similar (Roberts and Chaffee 1973). However, with both pyruvate + malate (South 1960) and glucose (South 1958) as substrate, the E_a values for hamster heart preparations were lower than those for rat preparations. Burlington and Wiebers (1967) showed similar results. These results may indicate that some enzyme or enzymes which participate in the oxidation from pyruvate + malate and glucose are responsible for these differences. The anaerobic glucolysis of brain slices from the ground squirrel (Citellus tridecemlineatus) had a lower Q_{10} between 5-16°C in hibernation compared with non-hibernating ground squirrels and rats. Kanugo and Prosser (1959 a and b) reported a higher Q_{10} for the oxygen consumption of warm acclimated goldfish (Carassius auratus) than for cold acclimated goldfish. Vroman and Brown (1963) found a significant difference in E_a of succinic dehydrogenase between rat (52.3 kJ/mole) and frog (18.4 kJ/mole). Woodard and Zimny (1973) examined the

same enzyme from hibernating and non-hibernating ground squirrels (Citellus tridecemlineatus). They found a lower E_a (11.7-38 kJ/mole) for the kidney but not for the heart or cerebrum of the hibernating animals compared with that of the non-hibernating animals. These findings are consistent with the hypothesis (South and House 1967) that the ability of a hibernator to maintain homeostasis at lowered body temperatures is related to lower enzymatic energies of activation and altered enzyme concentrations (Woodard and Zimny 1973).

Woodard and Zimny (1973) could not show any decrease in E_a of heart succinic dehydrogenase. This supports the above mentioned assumptions that some other enzyme in the oxidation of malate + pyruvate (South 1960) and glucose (South 1958) must be responsible for the difference in E_a observed by South (1958 and 1960) and Burlington and Wiebers (1967).

Because of this background, it was necessary to study the temperature dependence of the two important enzymes Lactic dehydrogenase (LDH) (EC 1.1.1.27) and Malic dehydrogenase (MDH) (EC 1.1.1.37) from homeotherms (guinea pigs), hibernators (hibernating and non-hibernating hedgehogs and bats), and poikilotherms (frogs and cod). These enzymes were selected because extensive studies of their seasonal variations in activity have been made on guinea pigs and hedgehogs (Olsson 1972 a).

Materials and Methods

Experimental animals

Adult animals of both sexes from the following species were used: hibernating and non-hibernating hedgehogs (Erinaceus europaeus), hibernating bats (Nyctalus noctula), guinea pigs (Cavia procellus), frogs (Rana temporaria) and cod (Gadus callarias). At least six animals of each species were used. All animals were captuered in the wild, except the guinea pigs, which were bred in the laboratory. The frogs and the hibernating bats were maintained in a room of 4°C (8 h light and 16 h darkness) for at least two months before the experiments and did not obtain any food. The cod were used immediately after the capture during the spring. The hibernating and non-hibernating hedgehogs were maintained and sampled as described before (Senturia et al., 1972).

Analysis of LDH and MDH activity and isoenzyme distribution

The organs (table 1) were homogenized according to Olsson (1972 a). These tissue homogenates were partly used for determination of LDH and MDH activity and partly for determination of LDH and MDH isoenzyme distribution. In both cases the same methods were used as described earlier (Olsson 1972 a). In order to study the enzyme activities of different temperatures, the spectrophotometric determinations were repeated with an interval of 5°C from 0°C to 65°C. The temperature in the cuvettes was always measured (Electric Thermometer TE3, CK1, Ellab) both before and after the enzymatic reactions. The temperatures did never differ more than one degree. The mean of these temperatures was used.

Analysis of data

The logarithms of the enzyme activities were plotted against the inverse temperature in degree Kelvin, according to Arrhenius (Precht et al., 1973). The log activity of -3 was transformed to $\pm$0. The equations of the lines, both for the calculation of the energy of activation (E_a kJ/mole) and the energy of inactivation (E_i kJ/mole) (table 1 and 2), were analysed by least-squares regression. Tests of significance were made between different pairs of energies of activation by conventional methods (Documenta Geigy 1970). Since the distribution of the five LDH isoenzymes from mammals is binomial for each cell line (Palmer and Karlsson 1969), the percentage of LDH-H activity was calculated according to Palmer and Karlsson (1969), and Karlsson and Palmer (1971). These LDH-H values from mammalian organs were plotted against the energy of activation and inactivation (fig 4 and 5). The activity of C-MDH (cytoplasmatic MDH) has also been plotted against the energy of activation (fig 6). The test of significance was made according to Spearman coefficient of rank correlation (Documenta Geigy 1970).
The points of intersection of the lines representing the energy of activation and inactivation for MDH were analysed according to Wilcoxon ranking sum test. The values for mammals were considered as one group and the values for non-mammals as the other group.

Results

The temperature dependence of both LDH and MDH are summarized in fig 1, 2 and 3 and table 1 and 2. The significance of the differences in the energy of activation between organs of the same species and between the same organs of different species are shown in table 3.

It is evident in this material (table 1 and 2) that the variation of the energy of activation is greater for LDH than for MDH in different organs and species. LDH, especially from heart and pectoralis muscle, is more thermostabile than MDH (fig 1 and 2).

The values for the heat of activation for mammalian LDH are significantly higher from the heart than from the liver (table 3). In many cases the values for the brain are significantly higher than those for the liver and significantly lower than those from the heart (table 3).

As can be seen from tables 1 and 4 the organs with high values for energy of activation also have a high percentage of LDH-H. The organs with high values for the energy of inactivation have high percentage of LDH-M. There is a positive correlation ($0.001<P<0.01$) between high values for the energy of activation and high activity of LDH-H (fig 4) and a negative correlation ($0.001<P<0.01$) (fig 5) between high values for the energy of inactivation and high activity of LDH-H (fig 5).

There are no significant differences between the hibernating and non-hibernating hedgehogs for the energy of activation of LDH (table 1 and 3). Also no significant differences in temperature dependence are seen between LDH from mammals and non-mammals (table 1 and 3).

The only significant differences are the low values for energy of activation from frog muscle and liver LDH, but not from frog heart,

cod heart or liver LDH. The heat lability of frog heart LDH is greater than cod heart and mammalian heart LDH. This difference in E_a of LDH from frog organs is correlated to the isoenzyme distribution (fig 8). The heart LDH of Rana temporaria consists of four bands: LDH_1, LDH_3, LDH_4 and LDH_5. The dominant band of both liver and skeletal muscle LDH is LDH_5.

The distribution of the LDH-isoenzymes from the various mammalian tissues is presented in table 4. The high percentage of LDH-H in brown adipose tissue, bat pectoralis muscle and guinea pig liver and kidney is remarkable. It is important to note that only heart, liver and pectoralis muscle show a strict binomial LDH-isoenzyme distribution. The temperature dependence in the Arrhenius plot for LDH is linear from $0^{\circ}C$ to $49^{\circ}C$.

The temperature dependence of MDH (table 2 and 3, fig 2 and 3) shows a significant difference in the temperature optimum between mammals and non-mammals. The temperature coordinate of the point of intersection of the two lines representing the energy of activation and inactivation differs significantly ($P < 0,01$) between mammals and non-mammals (table 2). This point of intersection is considered representative of the temperature of the activity maximum. If the heart MDH activity curves from fig 1 are placed in one single diagram (fig 3), it is obvious that the values from mammals and non-mammals have different temperature optima (Olsson 1972 a).

No correlation ($P > 0,05$) has been found between the energy of activation of MDH and the isoenzyme distribution (fig 6). No significant difference in the values for the energy of activation have been found between the hibernating and non-hibernating hedgehogs. No differences have been found in the values of activation energy of MDH between mammals and non-mammals (table 2 and 3).

The temperature dependence in the Arrhenius diagram is linear from 0°C to 40°C for the mammals and from 0°C to 30°C for the frog and cod.

The electrophoretic mobility of MDH-isoenzymes from guinea pig, hedgehog, bat and frog has been found to be different (fig 9). According to the anodal mobility of C-MDH the animals can be arranged in the following descending order: guinea pig, hedgehog, bat and frog, although the difference between guinea pig and hedgehog is small (Olsson 1972 a).

Discussion

The difference in the energy of activation of LDH is much greater between different organs from the same species than between the same organ from different species. This finding can be connected with changes in the isoenzyme distribution since it has been clearly shown that LDH-H has a greater energy of activation than LDH-M (fig 4). This is supported by Plagemann et al., (1961). In the same way, the differences in the energy of inactivation of LDH-H and LDH-M have been shown from fig 5. The same results have been found by Plagemann et al., (1961), Vesell and Yielding (1966), Karl and Peters (1967), Battelino et al., (1968) and Tollersrud (1970). According to these results, it is quite possible that small changes in the LDH-H/LDH-M ratio can cause changes in the energy of activation of the complete enzyme. Up to this date, no correlation has been reported between a change in the LDH-H/LDH-M ratio and a change in the energy of activation. In a previous work (Olsson 1972 a), the energy of activation was found to be maximal during prehibernation (autumn) and hibernation in hedgehog heart. The LDH-H/LDH-M ratio was minimal during hibernation and posthibernation (spring) (Olsson 1972 a). The significant correlation coefficient exists between these results (fig 7) ($0.01 < P < 0.05$). These changes have not been found in this study since the hedgehogs were not differentiated into summer, autumn, winter and spring animals but only into hibernating and non-hibernating animals. The increase in the energy of activation of LDH from hibernating hedgehog heart compared to the non-hibernating hedgehog and guinea pig is supported by the data of Johansson 1969. The differences in the temperature optima of LDH from heart, skeletal muscle and liver of guinea pig, non-hibernating and hibernating hedgehog observed by Johansson (1969) can be completely explained by (1). The

difference in the ratio LDH-H/LDH-M of each tissue; and (2) the difference in the energy of activation and inactivation of LDH-H and LDH-M.

Another seasonal rhythm of E_a has been observed by Audilet and Gray (1973) in glucose-6-phosphatedehydrogenase. The autumn group of Rana pipiens eggs and tadpoles had a higher E_a of G-6-PDH than the spring group.

The temperature dependence of frog LDH corresponds to that of mammalian LDH, as has been shown by Levy et al., (1971), Moyer et al., (1968), Goldberg and Wuntch (1967) and Wuntch and Goldberg (1968) that frog LDH-H is more inhibited by pyruvate than frog LDH-M. The LDH energy of activation is higher for frog heart than for frog liver and muscle. This may correspond to the high content of frog LDH-M in muscle and liver (fig 8). The energy of inactivation, however, is higher for frog heart than for frog muscle and liver LDH. Goldberg and Wuntch (1967) also found a higher thermostability in LDH from Rana pipiens muscle than from heart. No changes in the energy of activation of MDH have been found, nor is there any correlation between energy of activation and isoenzyme distribution.

These results show that there are no adaptive changes of differences in the energy of activation of LDH and MDH between hibernating and non-hibernating hedgehogs or between hedgehogs or guinea pigs which may be responsible for the lower temperature dependence of hibernators or poikilotherms. However, South (1958 and 1960), Covino and Hannon (1959), Vroman and Brown (1963) and Woodard and Zimny (1973) have reported differences in E_a between these animal groups.

There may be other kinetic differences of LDH and MDH explaining some enzymatic differences between these animal groups, such as the enzymatic temperature adaptation shown from poikilothermic animals (for review see Somero 1972). Somero's (1972) results are based mainly on temperature dependent changes in enzyme-substrate affinity ($1/K_m$).

By decreasing K_m of an enzyme at decreasing temperature, the Q_{10} of the enzyme activity will attain lower values than if K_m was constant or increasing.

This implies that the enzyme activity is more independent of temperature if K_m is directly proportional to the temperature and thus temperature may function as a positive modulator (Alkinson 1966).

The general picture of these studies (Somero 1972) is that cold adaptation of a poikilothermic animal shows a lower K_m temperature minimum than in the warm adapted animal. This has been found in LDH from the tropical and temperate genotype of the snake (Thamnophis sirtalis) (Aleksiuk 1971), pyruvate kinase from cold and warm adapted Alaskan king crab (Paralithodes camtschatica) (Somero 1969), isocitrate dehydrogenase from cold and warm adapted rainbow trout liver (Salmo gairdneri) (Moon and Hochachka 1971), and LDH from warm and cold living fishes (Salmo namaycush, Salvelinus frontinalis, Thynnus thynnus, Trematomus borchgrevinki, Lepidosiren paradoxa) (Hochachka and Somero 1968). In all cases the K_m temperature minima represent the living temperatures of the organisms.

"Three temporal classes of thermal adaptations have been observed in enzymes: (1), adaptation to immediate temperature change (Somero 1969 a, Somero and Hochachka 1969, Aleksiuk 1971), (2). adaptation to temperature change occurring over several days (Hochachka and Somero 1968, Baldwin and Hochachka 1970, Moon and Hochachka 1971), (3). adaptation to temperature on a phylogenetic time scale (Somero 1969 b, Aleksiuk 1971)". (Aleksiuk 1973). These adaptive changes were not all dependent on differences in isoenzyme distribution, but Beyer (1972) has shown other alternatives.

The values of E_a (activation energy) of LDH and MDH obtained in this study correspond well with the values of other animals. The energy of

activation of LDH and MDH from liver and skeletal muscle of platypus (Ornithorhynchus anatius) and echidna (Tachyglossus aculeatus) has been studied by Baldwin and Aleksiuk (1973). The values were 79 and 71 kJ/mole for LDH and 69 kJ/mole for MDH. The Arrhenius plots of V_{max} were linear from 10°C to 40°C for both enzymes. However, at substrate concentrations near K_m the activity was constant over the temperature range 25-35°C due to a decrease of K_m. Linear Arrhenius plots of cytoplasmatic MDH have also been found by Aleksiuk (1973) in the common crow (Corvus branchyrhynchos) (E_a = 50.6 kJ/mole) and in the pintail (Anas acuta) (E_a = 52.3 kJ/mole juv., 62.7 kJ/mole adult).

Hoskins and Aleksiuk (1973) examined liver supernatant MDH from cold and warm acclimated red sided garter snake (Thamnophis sirtalis parietalis) and found no change in the energy of activation (66.5 kJ/mole). Hochachka and Somero (1968) showed linear Arrhenius plots and similar activation energies (46-50 kJ/mole) of muscle LDH from some salmonids, blue fin Tuna (Thynnus thynnus), Antarctic fish (Trematomus borchgrevinki) and South American lungfish (Lepidosiren paradoxa). The minimal K_m values were found to correspond to the acclimation temperature of each species. Aleksiuk (1971) showed a difference in the Arrhenius plots of LDH from the cold and warm genotype of the reptile (Thamnophis sirtalis). The former had a linear Arrhenius plot with an energy of activation of 49.3 kJ/mole. The latter, however, had a break at 28°C, with an energy of activation of 64.8 kJ/mole above 28°C and 25 kJ/mole below 28°C. The two genotypes had different K_m minima (15°C and 35°C). This shows that LDH from this animal can compensate a temperature change in two ways: (1). by changes in K_m; and (2). by changes in E_a. Both factors are involved in the temperature adaptation of pyruvate kinase, which has been shown by Somero

and Hochachka (1968). K_m temperature minima were different from rainbow trout muscle (Salmo gairdnerii) (15°C), Trematomus muscle (5°C) and rat muscle (20°C). The Arrhenius plots of these animals were linear for rainbow trout (125 kJ/mole), but were broken for the other animals.

Rat muscle pyruvate kinase had an E_a of 41.8 kJ/mole above 25°C, but 83.6 kJ/mole below 25°C. However, Trematomus muscle pyruvate kinase, had an E_a of 83.6 kJ/mole above 10°C, but 41.8 kJ/mole below 10°C. The importance of these results is that both factors may be involved in the temperature adaptation of different enzymes, despite the critisism of Somero (1972) regarding the use of E_a. However, the results for LDH and MDH presented here do not show any temperature adaptation in E_a.

It has been shown that both LDH and MDH have linear Arrhenius plots from 0-30°C (table 1 and 2). Lenaz et al., (1972) has also shown this for MDH (54.3 kJ/mole). Lenaz et al., (1972) examined heart mitochondria Arrhenius plots of membrane bound enzymes from beef heart mitochondria: ATPase, betahydroxybutyric dehydrogenase, succinate oxidase, succinate-cytochrome-c-reductase. All of these showed breaks in the Arrhenius plots, except cytochrome-c-oxidase. Fumarase is a soluble matrix enzyme with a break (18.5°C) in the Arrhenius plot. The energy of activation above the breaking point was 69.8 kJ/mole and 32.6 kJ/mole below the breaking point. Lenaz et al., (1972) suggested that these break temperatures of membrane bound enzymes coincide with the transition temperatures of the phospholipids from a liquid-crystalline to a crystalline phase (Chapman 1969). McMurchie et al., (1973) correlated the change in heart rate at 21°C for the homeotherm with both temperature induced phase changes in the lipid components of membranes and the change in the activation energy of membrane

associated ATPases. None of these changes were observed in the membranes of the poikilothermic heart. These results indicate that there are differences in the temperature effects of the membranes and membrane-bound enzymes between poikilotherms and homeotherms.

The fact that LDH-H activity is more inhibited by high pyruvate concentrations than LDH-M activity (Plagemann et al., 1961, Cahn et al., 1962) has given rise to correlation of high LDH-H activity with an aerobic metabolism (Pfleiderer and Wachsmuth 1961, Cahn et al., 1962). Since Olsson (1972 a) and Burlington and Sampson (1968) have shown a decrease of the LDH-H/LDH-M ratio during hibernation it is necessary to examine the influence of temperature on the pyruvate inhibition. Plagemann et al., (1961) has shown that pyruvate inhibition increases with decreasing temperature. Interesting results have been reported by Somero (1973) with Gillichthys mirabilis muscle and by Cowey et al., (1969) with Pleuronectes platessa muscle. These fish muscle LDH showed an increasing pyruvate inhibition with decreasing temperature. This may be an adaptation to aerobic conditions when the water is cold and to anaerobic conditions when the water is warm. No temperature differentiating effect between LDH-H and LDH-M has been found. The difference in pyruvate inhibition measured at 25°C diminishes at 37°C (Vesell and Pool 1966), but Stambaugh and Post (1966) obtained a clear inhibition even at 37°C. Cowey et al., (1969) showed that beef LDH-M at 10°C has the same pyruvate inhibition as beef LDH-H at 35°C, which may imply that the temperature effect of the pyruvate inhibition of both LDH-H and LDH-M is the same. It may be as relevant at 10°C as it is at 25 or 37°C to discuss this theory of Pfleiderer and Wachsmuth (1961) and Cahn et al., (1962).

LDH showed a higher heat stability in the homeotherms than in the poikilotherms which always have lower body temperatures than homeo-

therms (table 2 and fig 3). Similar results with acetylcholine esterase were obtained by Johansson (1969).

The LDH isoenzymes (table 4) of pectoralis muscle of Nyctalus noctula contain predominantly LDH-H like other temperate vespertilionid bats (Manwell and Kerst 1966), and the large mastiff bat (Molossus fortis) (Valdivieso et al., 1968) as well as birds of long sustained flight (Wilson et al., 1963). But in the Jamaican fruit bat (Artibeus jamaicensis) and in the brown flower bat (Erophylla bombifrons) LDH-M predominates, which correlates well with their weak and irregular flying habits (Valdivieso et al., 1968). Another interesting observation (table 4) is the relatively high percentage of LDH-H from brown adipose tissue of hedgehog in comparison with white fat. This correlates well with the more aerobic metabolism of brown fat (Johansson 1959, Lindberg 1970, Olsson 1972 a and b).

The LDH-isoenzyme distribution of frog tissues has the same character as LDH from mammalian tissues, with one exception (fig 8). LDH_2 is completely missing in heart, which is supported by the work of Moyer et al., (1968). Chen (1968) examined both liver and muscle LDH from Rana temporaria and confirmed the existence of a dominant LDH_5.

The MDH isoenzymes of frog tissues show two bands from all the tissues in contrast to Chen (1968), who found only MDH band.

Different electrophoretic mobilities of C-MDH were obtained from guinea pig, hedgehog, bat and frog (fig 9). These findings correlate to the results of Kitto and Wilson (1966), who found the lowest electrophoretic mobilities of C-MDH from birds capable of natural hibernation. Paulsrud et al., (1970) correlated the high anodal mobility of C-MDH with a low value for the energy of activation of C-MDH, but this is not supported by the results from fig 6, as no correlation has been obtained between C-MDH and E_a of MDH from hedgehog and bat (Nyctalus noctula).

References

Adolph E.F. (1951) Responses to hypothermia in several species of infant mammals. Amer. J. Physiol. 166, 75 - 91.

Aleksiuk M. (1971) An isoenzymic basis for instantaneous cold compensation in reptiles: lactate dehydrogenase kinetics in Thamnophis sirtalis. Comp. Biochem. Physiol. 40B, 671 - 681.

Aleksiuk M. (1973) Temperature-dependent enzyme kinetics during avian ontogeny: malate dehydrogenase in the common crow (Corvus brachyrhynchos) and the pin-tail (Anas acuta). Can. J. Zool. 51, 557 - 565.

Atkinson D.E. (1966) Regulation of enzyme activity. Ann. Rev. Biochem. 35, 85 - 124.

Audilet D. O. & Gray J. (1973 a) Temperature influences in developing tadpoles. I. Influence of temperature on the enzymatic activity of glucoses-6-phosphate dehydrogenase. Cryobiology 10, 308 - 314.

Audilet D. O. & Gray J. (1973 b) Temperature influence in developing tadpoles. II. Influence of temperature on isozymes and energies of activation of partially purified glucose-6-phosphate dehydrogenase. Cryobiology 10, 315 - 320.

Baldwin J. & Hochachka P. W. (1970) Functional significance of isoenzymes in thermal acclimatization. Acetylcholinesterase from trout brain. Biochem. J. 116, 883 - 887.

Baldwin J. & Aleksiuk M. (1973) Adaptation of enzymes to temperature: lactate and malate dehydrogenases from platypus and echidna. Comp. Biochem. Physiol. 44 B, 363 - 370.

Battelino L. J., Jaime F. R. & Blanco A. (1968) Kinetic properties of rabbit testicular lactate dehydrogenase isozyme. J. biol. Chem. 243, 5185 - 5192.

Bowler K. & Duncan C. J. (1968 a) The temperature characteristics of the ATPases from a frog brain microsomal preparation. Comp. Biochem. Physiol. 24, 223 - 227.

Bowler K. & Duncan C. J. (1968b) The effect of temperature on the Mg^{2+} - dependent and Na^{+}-K^{+} ATPases of a rat brain microsomal preparation. Comp. Biochem. Physiol. 24, 1043 - 1054.

Bowler K. & Duncan C. J. (1969) The temperature characteristics of brain microsomal ATPases of the hedgehog: changes associated with hibernation. Physiol. Zool. 42, 211 - 219.

Burlington R. F. & Sampson J. H. (1968) Distribution and activity of lactic dehydrogenase isozymes in tissues from a hibernator and a non-hibernator. Comp. Biochem. Physiol. 25, 185 - 192.

Burlington R. F. & Wiebers J. E. (1967) The effect of temperature on glycolysis in brain and skeletal muscle from a hibernator and a non-hibernator. Phys. Biol. 40, 201 - 206.

Cahn R. D., Kaplan N. O., Levine L. & Zwilling E. (1962) Nature and development of lactic dehydrogenases. Science 136, 962 - 969.

Chapman D. (1969) Physical studies of lipid-lipid and lipid-protein interactions. Lipids 4, 251 - 260.

Chen P. S. (1968) Patterns of soluble proteins and multiple forms of dehydrogenases in Amphibian development. J. exp. Zool. 168, 337 - 350.

Covino B. G. & Hannon J. P. (1959) Myocardial metabolic and electrical properties of rabbits and ground squirrels at low temperatures. Amer. J. Physiol. 197, 494 - 498.

Cowey C. B., Luch J. E. & Knox D. (1969) Studies on crystalline lactate dehydrogenase from cardiac and skeletal muscle of plaice (Pleuronectes platessa) with particular reference to temperature. Biochim. biophys. Acta 191, 205 - 213.

Dawe A. R. & Landau B. R. (1960) The hibernating mammalian heart. Amer. Heart J. 59, 78 - 89.

Documenta Geigy (1970) Scientific Tables. 7th edition, 145 - 198. J. R. Geigy S. A. Basle, Switzerland.

Goldberg E. & Wuntch T. (1967) Electrophoretic and kinetic properties of Rana pipiens lactate dehydrogenase isozymes. J. exp. Zool. 165, 101 - 110.

Hannon J. P. (1958) Effect of temperature on the heart rate, electrocardiogram and certain myocardial oxidations of the rat. Circulat. Res. 6, 771 - 778.

Hannon J. P., Beyer R. E., Burlington R. F., Somero G. N. & Bland J. H. (1972) A guide for future studies of low temperature metabolic function. In: Hibernation - Hypothermia, perspectives and challenges (Edited by South F. E., Hannon J. P., Willis J. R., Pengelley E. T. & Alpert N. R. 99 - 120, Elsevier, Amsterdam.

Hochachka P. W. & Somero G. N. (1968) The adaptation of enzymes to temperature Comp. Biochem. Physiol. 27, 659 - 668.

Hochachka P. W. & Somero G. N. (1971) Biochemical adaptation to the environment. In Fish Physiology. (Edited by Hoar W. S. & Randall P. J.) vol. VI, 99 - 156, Academic Press, New York.

Hoskins M. A. H. & Aleksiuk M. (1973) Effects of temperature on the kinetics of malate dehydrogenase from a cold climate reptile. Thamnophis sirtalis parietalis. Comp. Biochem. Physiol. 45B, 343 - 353.

Johansson B. W. (1959) Brown fat: a review. Metabolism 8, 221 - 240

Johansson B. W. (1967) Heart and circulation in hibernators. In Mammalian Hibernation III (Edited by Fisher K. C., Dawe A. R., Lyman C. P., Schönbaum E. & South F. E.) 200 - 218, Oliver & Boyd, Edinburgh.

Johansson B. W. (1969 a) Electrocardiographic changes in depressed metabolism in Depressed Metabolism. (Edited by Mussacchia X.J. & Saunders J.F.), 313-374. Elsevier, New York.

Johansson B. W. (1969 b) Temperaturedependence of cholinesterase and lactate dehydrogenase in the guinea pig, hedgehog and codfish. Acta physiol. scand. 77, 1 - 6.

Kanugo M. S. & Prosser C. L. (1959 a) Physiological and biochemical adaptation of goldfish to cold and warm temperatures. I. Standard and active oxygen consumptions of cold and warm acclimated goldfish at various temperatures. J. cell. comp. Physiol. 54, 259 - 263.

Kanugo M. S. & Prosser C. L. (1959 b) Physiological and biochemical adaptation of goldfish to cold and warm temperatures. II. Oxygen consumption of liver homogenate; oxygen consumption and oxidative phosphorylation of liver mitochondria. J. cell. comp. Physiol. 54, 265 - 274.

Karl W. F. & Peters T. (1967) Thermal stability of lactic dehydrogenase from human tissues. Am. J. clin. Path. 47, 171 - 174.

Kitto G. B. & Wilson A. C. (1966) Evolution of malate dehydrogenase in birds. Science (N.Y.) 153, 1408 - 1410.

Karlsson B. W. & Palmer L. S. (1971) Lactic dehydrogenase isoenzyme distribution in various tissue fractions of the developing mammalian liver. Comp. Biochem. Phsyiol. 38B, 299 - 308.

Kayser C. (1957) Physiological aspects of hypothermia. Ann. Rev. Physiol. 19, 83 - 120.

Lenaz G., Sechi A. M., Parenti-Castelli G., Landi L. & Bertoli E. (1972) Activation energies of different mitochondrial enzymes: breaks in Arrhenius plots of membrane-bound enzymes occur at different temperatures. Biochem. biophys. Res. Commun. 49, 536 - 542.

Levy P. L., Kaplan R. H., Wejksnora P. J., Drewry G. E. and Salthe S. N. (1971) Lactate dehydrogenases in the frog, Eleutherodactylus coqui. Comp. Biochem. Physiol. 39B, 1053 - 1057.

Lindberg O. ed. (1970) Brown Adipose Tissue. 337 pages. Elsevier, New York.

Lyman C. P. & Blinks D. C. (1959) The effect of temperature on the isolated hearts of closely related hibernators and non-hibernators. J. cell. comp. Physiol. 54, 53 - 64.

Lyons J. M. & Raison J. K. (1970) A temperature-induced transition in mitochondrial oxidation: contrasts between cold and warm-blooded animals. Comp. Biochem. Physiol. 37, 405 - 411.

Manwell C. & Kerst K. V. (1966) Possibilities of biochemical taxonomy of bats using hemoglobin, lactate dehydrogenase, esterases and other proteins. Comp. Biochem. Physiol. 17, 741 - 754.

McMurchie E. J., Raison J. K. and Cairncross K. D. (1973) Temperature-induced phase changes in membranes of heart: a contrast between the thermal response of poikilotherms and homeotherms. Comp. Biochem. Physiol. 44B, 1017 - 1026.

Mokrash L. C., Grady H. J. & Grisolia S. (1960) Thermogenic and adaptive mechanisms in hibernation and arousal from hibernation. Amer. J. Physiol. 199, 945 - 949.

Moon T. W. & Hochachka P. W. (1971) Temperature and enzyme activity in poikilotherms. Isocitrate dehydrogenases in rainbow-trout liver. Biochem. J. 123, 695 - 705.

Moyer F. H., Speaker C. B. & Wright D. A. (1968) Characteristics of lactate dehydrogenase isoenzymes in Amphibians. Ann. N.Y. Acad. Sci. 151, 650 - 669.

Olsson S-O. R. (1972 a) Dehydrogenases (LDH, MDH, G-6-PDH and alfa-GPDH) in the heart, liver, white and brown fat. In Seasonal variations in the physiology and biochemistry of the European hedgehog (Erinaceus europaeus) including comparisons with non-hibernators, guinea pig and man. (Edited by Johansson B. W. & Senturia J. B.) Acta physiol. scand. Suppl. 380, 62 - 95.

Olsson S-O. R. (1972 b) Ultrastructure of brown adipose tissue and myocardium. Ibidem. 117 - 130.

Palmer L. S. & Karlsson B. W. (1969) Lactic dehydrogenase isozyme distributions in various cell types of the mammalian liver. Can. J. Biochem. 47, 1171 - 1178.

Paulsrud J. R., Mann K. G. & Dryer R. L. (1970) A comparison of rat and bat malic dehydrogenase isoenzymes. In Brown Adipose Tissue. (Edited by Lindberg O.) Chap. 8. 197 - 206. Elsevier. New York.

Pfleiderer G. & Wachsmuth E. D. (1961) Alters-und funktionsabhängige Differenzierung der Laktatdehydrogenase menschlicher Organe. Biochem. Z. 334, 185 - 198.

Plagemann P. G. W., Gregory K. F. & Wroblewski F. (1961) Die elektrophoretisch trennbaren Lactat-dehydrogenasen des Säugetieres. III. Einfluss der Temperatur auf die Lactat-dehydrogenasen des Kaninchens. Biochem. Z. 334, 37 - 48

Precht H., Christophersen J., Hensel H. & Larcher W. (1973) Temperature and life. Springer Verlag, Berlin.

Raison J. K., Lyons J. M., Mehlhorn R. J. & Keith A. D. (1971 a) Temperature-induced phase changes in mitochondrial membranes detected by spin-labelling. J. biol. Chem. 246, 4036 - 4040.

Raison J. K., Lyons J. M. & Thomson W. W. (1971 b) The influence of membranes on the temperature-induced changes in the kinetics of some respiratory enzymes of mitochondria. Arch. Biochem. 142, 83 - 90.

Robert J. C. & Chaffee R. R. J. (1973) Effects of cold acclimation, hibernation and temperature on succinoxidase activity of heart homogenates from hamster, rat and squirrel monkey. Comp. Biochem. Physiol. 44B, 137 - 144.

Senturia J. B., Eklund B. & Johansson B. W. (1972) General methods and materials. In Seasonal variations in the physiology and biochemistry of the European hedgehog (Erinaceus europaeus) including comparisons with non-hibernators, guinea-pig and man. (Edited by Johansson B. W. & Senturia J. B.) Acta physiol. scand. Suppl. 380, 15 - 20.

Senturia J. B., Steward S. & Menaker M. (1970) Rate temperature relationships in the isolated hearts of ground squirrels. Comp. Biochem. Physiol. 33, 43 - 50.

Smith A. U. (1957) Problems in the resuscitation of mammals from body temperatures below 0°C. Proc. roy. Soc. B. 147, 533 - 544.

Somero G. N. (1969 a) Pyruvate kinase variants of the Alaskan King-Crab. Evidence for a temperature-dependent interconversion between two forms having distinct and adaptive kinetic properties. Biochem. J. 114, 237 - 241.

Somero G. N. (1969 b) Enzymic mechanisms of temperature compensation: immediate and evolutionary effects of temperature on enzymes of aquatic poikilotherms. Am. Nat. 103, 517 - 530.

Somero G. N. (1972) Molecular mechanisms of temperature compensation in aquatic poikilotherms. In Hibernation-Hypothermia, perspectives and challenges. (Edited by South F. E., Hannon J. P., Willis J. R., Pengelley E. T., Alpert N. R.) 55 - 80. Elsevier. Amsterdam.

Somero G. N. (1973) Thermal modulation of pyruvate metabolism in the fish Gillichtys mirabilis: the role of lactate dehydrogenases. Comp. Biochem. Physiol. 44B, 205 - 209.

Somero G. N. & Hochachka P. W. (1968) The effect of temperature on catalytic and regulatory functions of pyruvate kinases of the rain-bow trout and the Antarctic fish Trematomus bernacchii. Biochem. J. 110, 395 - 400.

Somero G. N. & Hochachka P. W. (1969) Isoenzymes and short term temperature compensation in poikilotherms: Activation of lactate dehydrogenase isoenzymes by temperature decreases. Nature 223, 194 - 195.

South F. E. (1958) Rates of oxygen consumption and glycolysis of ventricle and brain slices, obtained from hibernating and non-hibernating mammals, as function of temperature. Physiol. Zool. 31, 6 - 15.

South F. E. (1960 a) Hibernation, temperature and rates of oxidative phosphorylation by heart mitochondria. Amer. J. Physiol. 198, 463 - 466.

South F. E. (1960 b) Some metabolic specializations in tissues of hibernating mammals. In Mammalian Hibernation. (Edited by Lyman C. P. & Dawe A. R.) Bull. Mus. comp. Zool. (Harv.) 124, 475 - 492.

South F. E. & House W. A. (1967) Energy metabolism in hibernation. In Mammalian Hibernation III. (Edited by Fisher K. C., Dawe A. R., Lyman C. P., Schönbaum E. & South F. E.) 305 - 324. Oliver & Boyd, Edinburgh.

Stambaugh R. & Post D. (1966) Effects of tissue extracts and temperature on lactate dehydrogenase isozymes. Biochim. biophys. Acta 122, 541 - 543.

Tollersrud S. (1970) Heat stability of serum lactate dehydrogenase and its isoenzymes in young and adult cattle and sheep. Acta vet. scand. 11, 510 - 524.

Valdivieso D., Conde E. & Tamsitt J. R. (1968) Lactate dehydrogenase studies in Puerto Rican bats. Comp. Biochem. Physiol. 27, 133 - 138.

Vesell E. S. & Pool P. E. (1966) Lactate and pyruvate concentrations in exercised ischemic canine muscle: relationship of tissue substrate level to lactate dehydrogenase isozym pattern. Proc. nat. Acad. Sci. 55, 756 - 762.

Vesell E. S. & Yielding K. L. (1966) Effects of pH ionic strength and metabolic intermediates on the rates of heat inactivation of lactate dehydrogenase isozymes. Proc. nat. Acad. Sci. 56, 1317 - 1324.

Vroman H. E. & Brown J. R. C. (1963) Effect of temperature on the activity of succinic dehydrogenase from the livers of rats and frogs. J. cell. comp. Physiol. 6, 129 - 131.

Wilson A. C., Cahn R. D. & Kaplan N. O. (1963) Functions of the two forms of lactic dehydrogenase in the breast muscle of birds. Nature (Lond.) 197, 331 - 334.

Woodard C. B. & Zimny M. L. (1973) Energy of activation of succinic dehydrogenase in a hibernator. Amer. J. Physiol. 208, 1247-1252

Wuntch T. & Goldberg E (1968) Physico-chemical properties of Rana pipiens lactate dehydrogenase. Ann. N.Y. Acad. Sci. 151, 670 - 671.

Zimny M. L. & Taylor S. (1965) Cardiac metabolism in the hypothermic ground squirrel and rat. Amer. J. Physiol. 208, 1247 - 1252.

Table 1

Arrhenius plots of LDH V_{max}.

The equations are calculated by the least-squares regression between 0 - 49°C for E_a and between 49 - 65°C for E_i

	Energy of activation (E_a)				Energy of inactivation (E_i)				
	Equation	E_a (kJ/mole	r^{**}	N	Equation	E_i (kJ)	r^{**}	N	LDH-H %
Heart Hedgehog Hib.	y = 14.57-3.76 x	72.0	-0.990	41	y = 3.06±0.04 *	0		15	76.2
Brain " "	y = 13.70-3.53 x	67.6	-0.986	42	y = 0.88x-0.004	16.9	0.540	13	44.9
Liver " "	y = 12.12-2.98 x	57.0	-0.992	42	y = 5.39x-13.69	103.1	0.912	18	2.4
Heart " Non H	y = 14.89-3.90 x	74.6	-0.974	69	y = 2.98 ±0.05 *	0		16	79.1
Brain " "	y = 12.21-3.12 x	59.6	-0.942	66	y = 3.24x-7.14	62.0	0.825	16	48.0
Liver " "	y = 12.26-3.03 x	58.0	-0.979	71	y = 17.15x-49.71	328.1	0.889	16	0.6
Heart Bat Hib.	y = 15.64-4.14 x	79.2	-0.998	40	y = 3.03±0.07 *	0		12	82.6
M.pectoralis Bat "	y = 14.89-3.92 x	75.0	-0.996	44	y = 2.97±0.09 *	0		12	77.1
Liver " "	y = 12.51-3.00 x	57.4	-0.994	44	y = 12.74x-35.50	243.8	0.856	12	9.7
Heart Guinea-pig	y = 13.53-3.49 x	66.9	-0.991	44	y = 2.89±0.05 *	0		11	80.6
Brain "	y = 11.80-3.04 x	58.2	-0.982	44	y = 1.02x-0.54	19.5	0.551	12	75.3
Liver "	y = 10.79-2.72 x	52.0	-0.982	44	y = 6.22x-16.41	119.0	0.880	12	49.1
Heart Frog	y = 14.22-3.64 x	69.6	-0.989	44	y = 5.01x-12.23	96.0	0.770	12	
Muscle "	y = 10.88-2.51 x	48.1	-0.990	35	y = 1.74x-2.45	33.3	0.904	12	
Liver "	y = 10.25-2.54 x	48.6	-0.973	44	y = 2.10x-4.05	40.3	0.919	12	
Heart cod	y = 12.27-3.28 x	62.7	-0.974	36	y = 2.15±0.03 *	0		12	
Liver "	y = 12.95-3.59 x	68.7	-0.989	44	y = 1.87±0.03 *	0		12	

* ± SD ** coefficient of correlation

Table 2

Arrhenius plots of LDH V_{max}. The equations are calculated by the least-squares regression for E_a between 0-40°C (the mammals) and between 0-30°C (the frog and cod) and for E_i between 55-65°C (the mammals) and between 44-65°C (the frog and cod).

	Energy of activation (E_a), Equation	E_a kJ/mole	r *	n	Energy of inactivation (E_i) Equation	E_i kJ/mole	r *	n	Point of intersection between E_a and E_i
Heart Beisehog hib	y = 11.21-2.64 x	[illegible]	-0.964	44	y = 8.50x-23.04	169.1	0.819	20	3.079
Brain " "	y = 11.91-2.96 x	58.7	[illegible]	42	y = 15.55x-58.27	229.2	0.853	10	3.075
Liver " "	y = 11.25-2.74 x	52.1	[illegible]	34	y = 15.54x-44.77	291.4	0.820	12	3.063
Heart " non-hib	y = 11.75-2.85 x	54.5	[illegible]	40	y = 14.18x-40.28	264.4	[illegible]	15	3.075
Brain " "	y = 10.95-2.61 x	51.1	[illegible]	31	y = 15.44x-50.81	286.7	[illegible]	17	3.080
Liver " "	y = 10.61-2.58 x	49.3	-0.959	72	y = 13.04x-43.53	287.7	0.838	16	3.074
Heart Bat hib	y = 12.77-3.02 x	[illegible]	[illegible]	55	y = 16.04x-48.42	298.5	[illegible]	12	3.080
M pecto-ralis " "	y = 12.59-2.96 x	56.1	-0.988	56	y = 21.8 x-81.68	335.4	0.900	12	3.091
Liver " "	y = 13.26-3.17 x	60.6	-0.994	36	y = 8.55x-23.06	168.9	0.941	10	3.100
Heart Guinea pig	y = 12.93-3.17 x	60.6	-0.991	35	y = 18.39x-53.25	381.9	0.852	11	3.070
Brain "	y = 11.87-2.93 x	56.1	-0.986	34	y = 16.23x-46.97	310.6	0.942	11	3.071
Liver "	y = 11.73-2.89 x	55.3	-0.989	35	y = 20.04x-58.68	383.6	0.956	12	3.070
Heart Frog	y = 12.27-2.93 x	56.1	-0.990	28	y = 18.21x-54.69	348.4	0.917	15	3.161
Muscle "	y = 11.12-2.75 x	52.7	-0.985	27	y = 10.24x-49.04	210.8	0.746	15	3.168
Liver "	y = 11.77-2.87 x	54.8	-0.976	26	y = 7.44x-21.33	142.3	0.884	20	3.210
Heart Cod	y = 10.45-2.81 x	53.7	-0.916	28	y = 14.68x-44.58	280.9	0.821	12	3.152
Liver "	y = 8.48-2.27 x	43.5	-0.954	28	y = 17.17x-52.70	328.6	0.956	12	3.148

* Coefficient of correlation.

Table 3

Tests of significance between: (1). the differences in E_a LDH from different tissues and animals and (2). between the differences in E_a MDH from different tissues and animals. Levels of significance: * $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $P < 0.001$

	Heart Hedgehog hib		Brain Hedgehog hib		Liver Hedgehog hib		Heart Hedgehog non-hib		Brain Hedgehog non-hib		Liver Hedgehog non-hib		Heart Bat hib		M pectoralis Bat hib		Liver Bat hib	
	LDH	MDH	LDH	MDH	LDH	MDH	LDH	MDH	LDH	MDH	LDH	MDH	LDH	MDH	LDH	MDH	LDH	MDH
Heart Hedgehog hib																		
Brain " "	NS	NS																
Liver " "	***	NS	***															
Heart " non-hib	NS	NS																
Brain " "			*	NS			***	NS										
Liver " "					NS	NS	***	NS	NS	NS								
Heart Bat hib	***	***					NS	NS										
M pectoralis " "													***	NS				
Liver " "					NS	***					NS	***	***	NS	***	*		
Heart Guinea pig	*	***					**	NS					***	NS				
Brain "			***	NS					NS	NS								
Liver "					*	NS					**	NS					***	***
Heart Frog	NS	NS					NS	NS					***	NS				
Muscle "															***	NS		
Liver "					***	NS					***	NS					***	*
Heart Cod	***	NS					***	NS					***	NS				
Liver "					***	*					***	NS					***	***

Table 3 cont

	Heart Guinea pig		Brain Guinea pig		Liver Guinea pig		Heart Frog		Muscle Frog		Liver Frog		Heart Cod		Liver Cod	
	LDH	MDH	LDH	MDH	LDH	MDH	LDH	MDH	LDH	MDH	LDH	MDH	LDH	MDH	LDH	MDH
Heart Guinea pig																
Brain "	***	*														
Liver "	***	**	**	NS												
Heart Frog	NS	*			LDH	MDH										
Muscle "							***	NS								
Liver "					NS	NS	***	NS	NS	NS						
Heart Cod	NS	NS					*	NS			LDH	MDH				
Liver "					***	***					***	**	*	NS		
															LDH	MDH

Table 4

Percental activity of the isoenzymes, presented as means ± S.D.

	Number of animals	LDH-H	LDH-1	LDH-2	LDH-3	LDH-4	LDH-5	C-MDH	M-MDH
Erinaceus europaeus (Hedgehog)	6								
Summer									
Heart		79.1	44.8 ±4.6	39.0 ±2.9	10.0 ±1.5	0.1 ±0.3	6.0 ±2.5	59.0 ±7.3	40.4 ±7.2
Brain		48.0	12.4 ±1.8	24.9 ±1.6	28.3 ±1.5	11.0 ±1.3	23.6 ±3.3	52.8 ±7.7	47.2 ±7.7
Liver		0	0	0	0	0	99.9 ±0.4	55.8 ±8.7	42.7 ±8.0
Muscle		38.2	12.1 ±8.2	19.0 ±4.6	17.9 ±1.0	11.5 ±3.3	39.9 ±9.9	59.9 ±4.6	40.1 ±4.6
Kidney		38.5	16.0 ±7.7	20.1 ±8.0	11.7 ±4.8	6.1 ±2.5	47.3 ±14.0	51.0 ±4.5	48.5 ±3.4
White fat		11.8	0.9 ±2.5	3.5 ±2.9	12.4 ±7.3	8.4 ±6.3	73.8 ±15.5	37.5 ±18.2	62.5 ±18.2
Brown fat		37.8	2.3 ±0.5	21.0 ±1.7	31.4 ±2.2	16.1 ±2.3	29.1 ±2.5	42.1 ±3.8	57.9 ±3.8
Erinaceus europaeus (Hedgehog)	8								
Hibernating									
Heart		76.2	41.4 ±1.9	37.8 ±3.6	12.7 ±2.5	0.4 ±0.5	7.4 ±3.5	53.7 ±6.1	46.3 ±6.1
Brain		44.9	12.2 ±1.0	21.7 ±1.4	27.3 ±2.3	11.2 ±1.3	27.5 ±4.0	63.8 ±9.5	36.1 ±9.5
Liver		2.4	0	0	3.6 ±2.6	2.3 ±2.5	93.9 ±4.9	65.5 ±5.7	34.5 ±5.7
Muscle		49.0	19.6 ±6.0	21.4 ±3.0	21.6 ±3.4	10.2 ±5.4	27.0 ±4.5	64.0 ±1.0	36.0 ±1.0
Kidney		40.3	15.2 ±3.5	22.4 ±5.9	13.0 ±1.2	7.2 ±1.9	43.6 ±9.8	62.5 ±16	37.5 ±16
Nyctalus noctula (Bat)	6								
Heart		82.0	51.2 ±2.2	32.5 ±1.5	10.5 ±1.5	4.7 ±0.6	1.1 ±1.6	33.2 ±3.5	68.6 ±6.0
Liver *		9.7	0.2 ±0.4	0.1 ±0.2	1.2 ±0.4	35.2 ±4.1	58.2 ±3.0	45.5 ±4.0	51.0 ±3.3
M. pectoralis		77.1	53.6 ±7.6	26.1 ±2.8	5.8 ±0.4	4.2 ±2.0	10.3 ±8.7	44.5 ±4.0	55.3 ±4.0

* = Nyctalus noctula Liver — LDH-6: 5.2 ±1.0

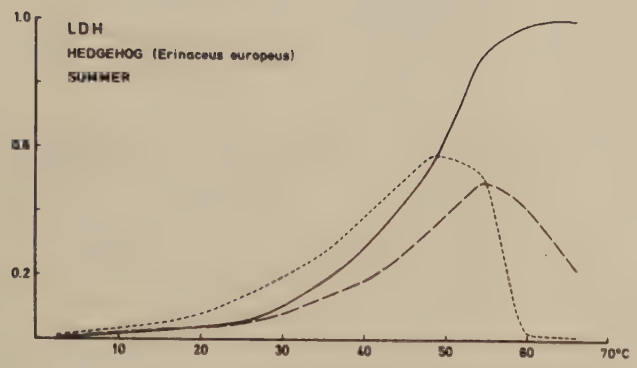
Table 4 cont

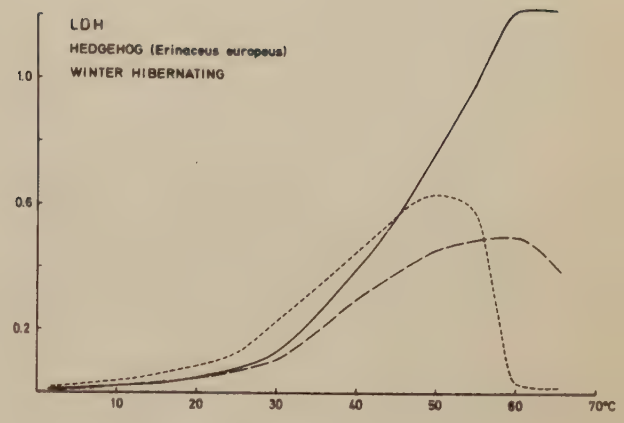
	Number of animals	LDH-H	LDH-1	LDH-2	LDH-3	LDH-4	LDH-5	C-MDH	M-MDH
Cavia porcellus (Guinea pig)	2								
Heart		80.0	53.8 ±5.4	24.3 ±1.7	13.0 ±1.4	5.8 ±1.0	2.0 ±1.2	-	-
Brain		73.3	41.8 ±6.8	25.0 ±0.5	20.3 ±1.9	10.3 ±2.2	1.5 ±1.0	-	-
Liver		49.1	9.5 ±2.1	22.5 ±3.1	32.5 ±3.0	25.8 ±0.5	5.5 ±2.4	-	-
Muscle		26.1	16.0 ±1.4	3.5 ±0.7	8.5 ±0.7	13.0 ±0	58.5 ±3.5	-	-
Kidney		71.5	43.0 ±2.8	22.5 ±3.5	18.0 ±1.4	10.5 ±2.1	3.0 ±3.5	-	-

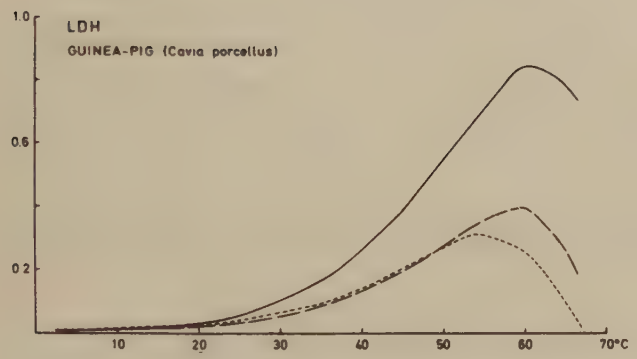
Figure 1

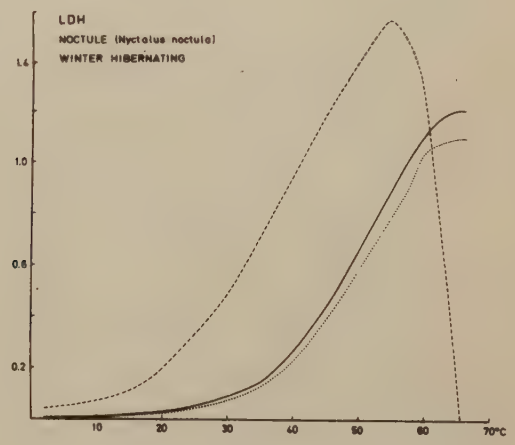
The temperature dependence of LDH V_{max} from heart, liver, brain and skeletal muscle from (a) non-hibernating hedgehog, (b) hibernating hedgehog, (c) guinea pig, (d) hibernating bat, (e) frog and (f) cod. The lines represent the mean curves from at least 6 animals/diagram. The axis of ordinates: activity/wet weight. The axis of abscissas: temperature in degree Celsius.

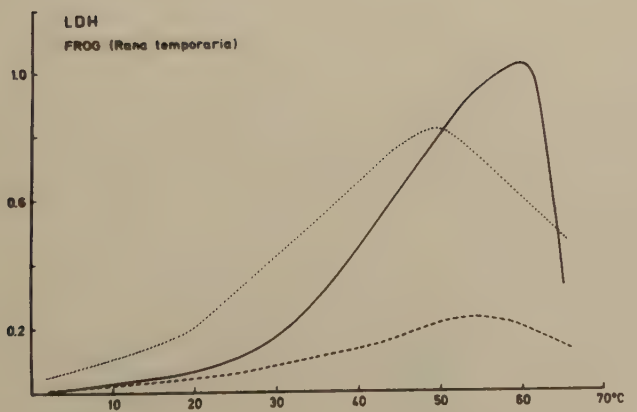
Fig 1

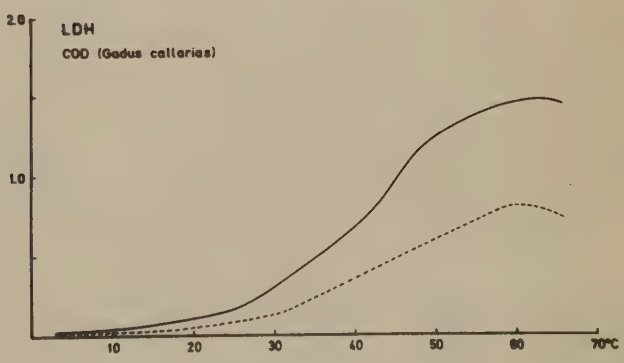










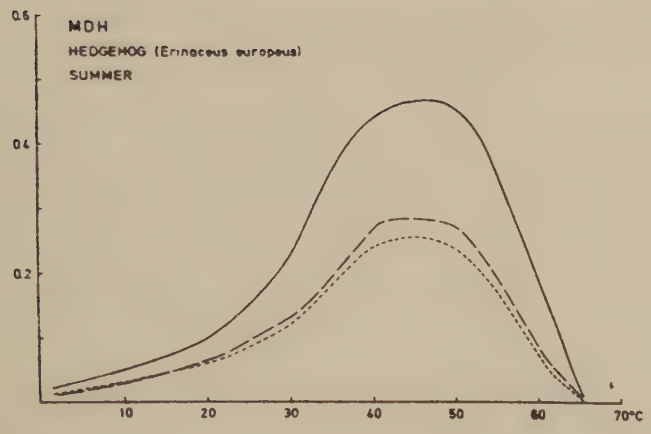


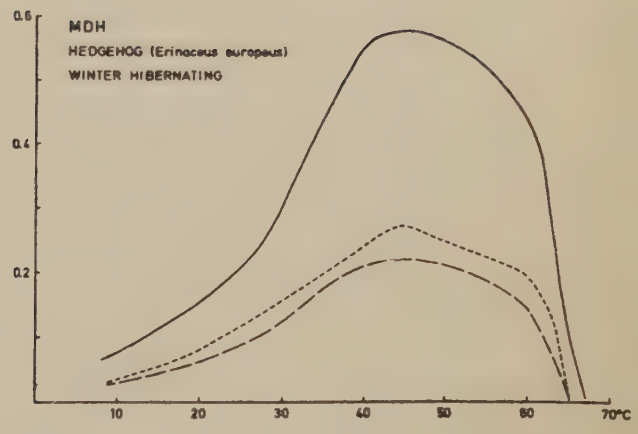
HEART
LIVER
BRAIN
MUSCLE

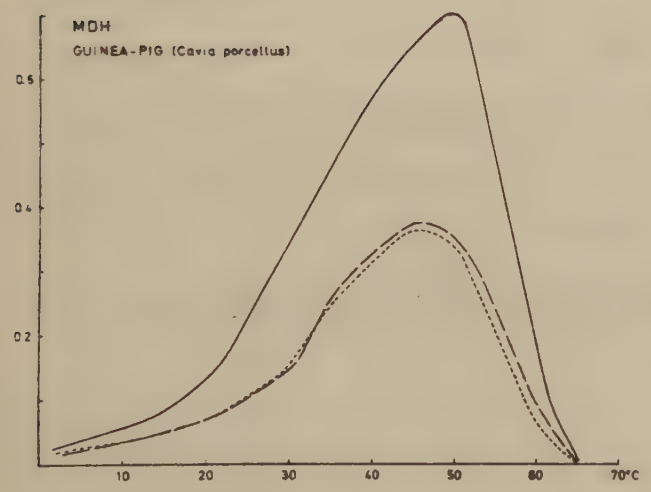
Figure 2

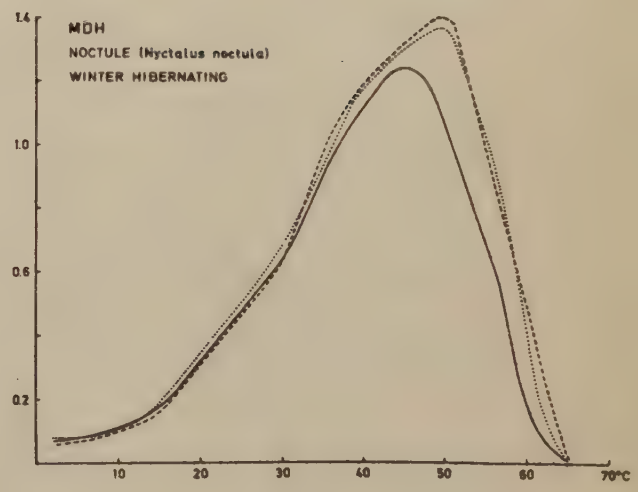
The temperature dependence of MDH V_{max} from heart, liver, brain and skeletal muscle from at least 6 animals/diagram from (a) non-hibernating hedgehog, (b) hibernating hedgehog, (c) guinea pig, (d) hibernating bat, (e) frog and (f) cod.

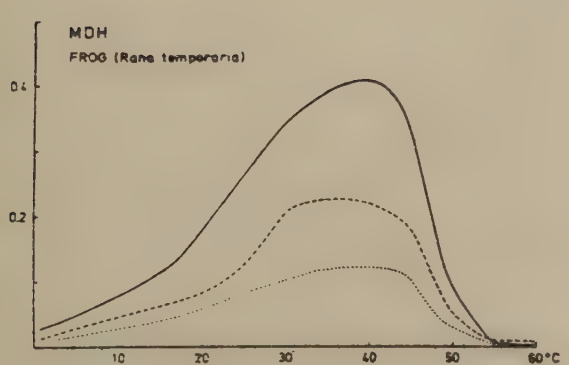
The axis of ordinates: activity/wet weight. The axis of abscissas: temperature in degree Celsius.


MDH
HEDGEHOG (Erinaceus europeus)
SUMMER
0.6
0.4
0.2
10
20
30
40
50
60
70°C


MDH
HEDGEHOG (Erinaceus europeus)
WINTER HIBERNATING
0.6
0.4
0.2
10
20
30
40
50
60
70°C


MDH
GUINEA-PIG (Cavia porcellus)
0.5
0.4
0.2
10
20
30
40
50
60
70°C


MDH
NOCTULE (Nyctalus noctula)
WINTER HIBERNATING
1.4
1.0
0.6
0.2
10
20
30
40
50
60
70°C


MDH
FROG (Rana temporaria)
0.4
0.2
10
20
30
40
50
60°C

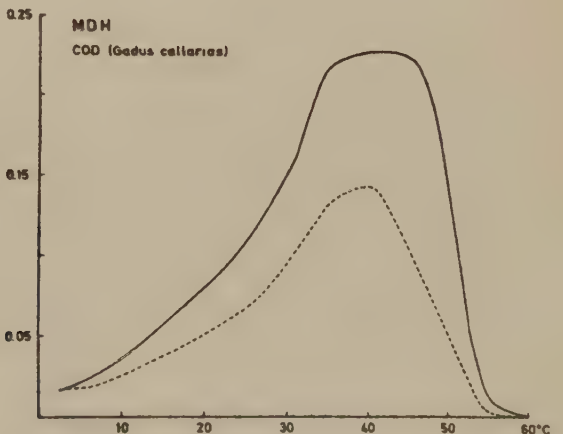
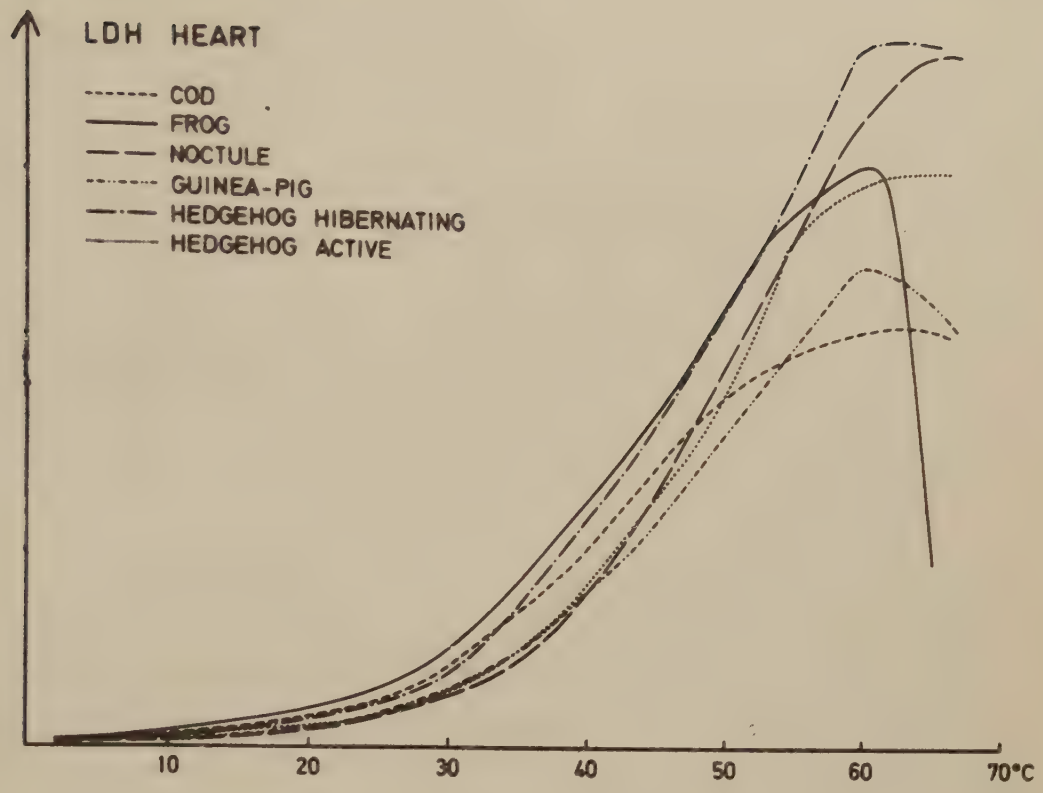

MDH
COD (Gadus callarias)
0.25
0.15
0.05
10
20
30
40
50
60°C

Figure 3

The temperature dependence of (a) LDH V_{max} and (b) MDH V_{max} from the hearts of cod, frog, bat, guinea pig, hibernating and non-hibernating hedgehog. These curves have been taken from fig 1 and 2 to illustrate (1) the differences between different animals and (2) the differences between LDH and MDH. The scale of the axis of ordinates is not the same for bat and cod as from the other animals.

The axis of ordinates: activity/wet weight. The axis of abscissas: temperature in degree Celsius.


LDH HEART
COD
FROG
NOCTULE
GUINEA-PIG
HEDGEHOG HIBERNATING
HEDGEHOG ACTIVE
10
20
30
40
50
60
70°C

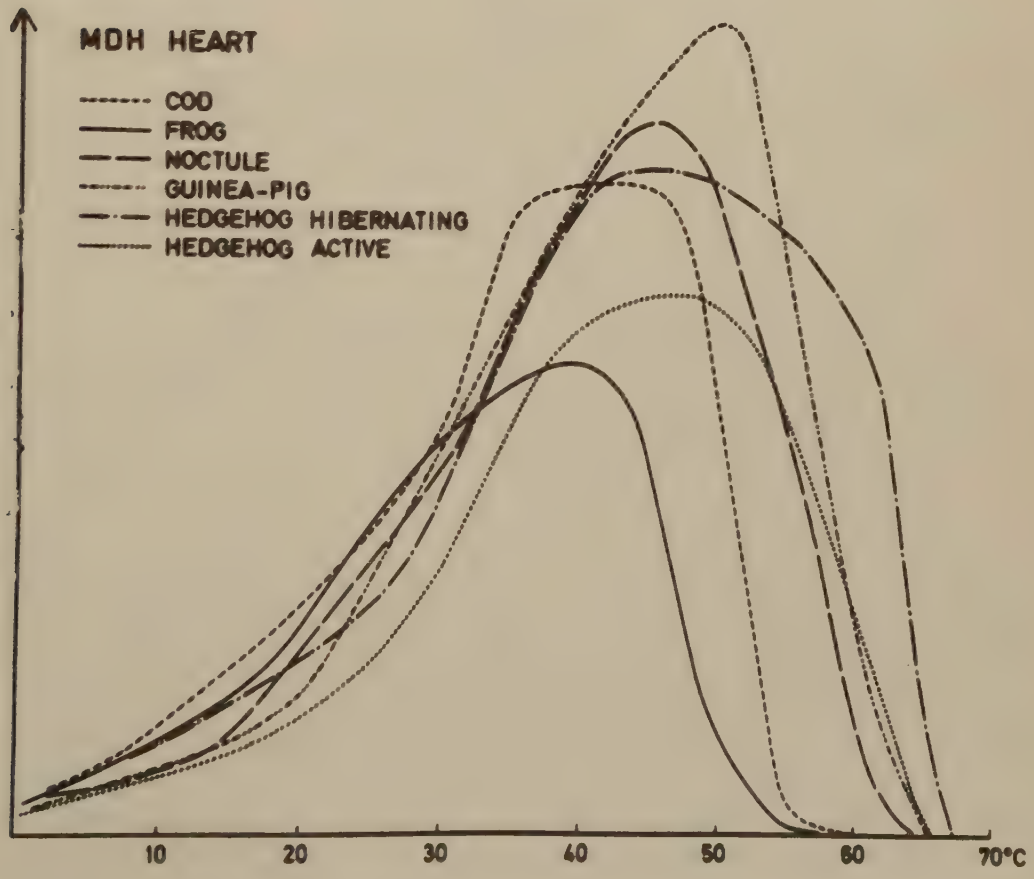

MDH HEART
COD
FROG
NOCTULE
GUINEA-PIG
HEDGEHOG HIBERNATING
HEDGEHOG ACTIVE
10
20
30
40
50
60
70°C

Figure 4

Correlation between the percentage LDH-H (the axis of abscissas) and the energy of activation E_a of LDH (the axis of ordinates) from the results presented in table 1 and 4.

LDH

ENERGY OF ACTIVATION

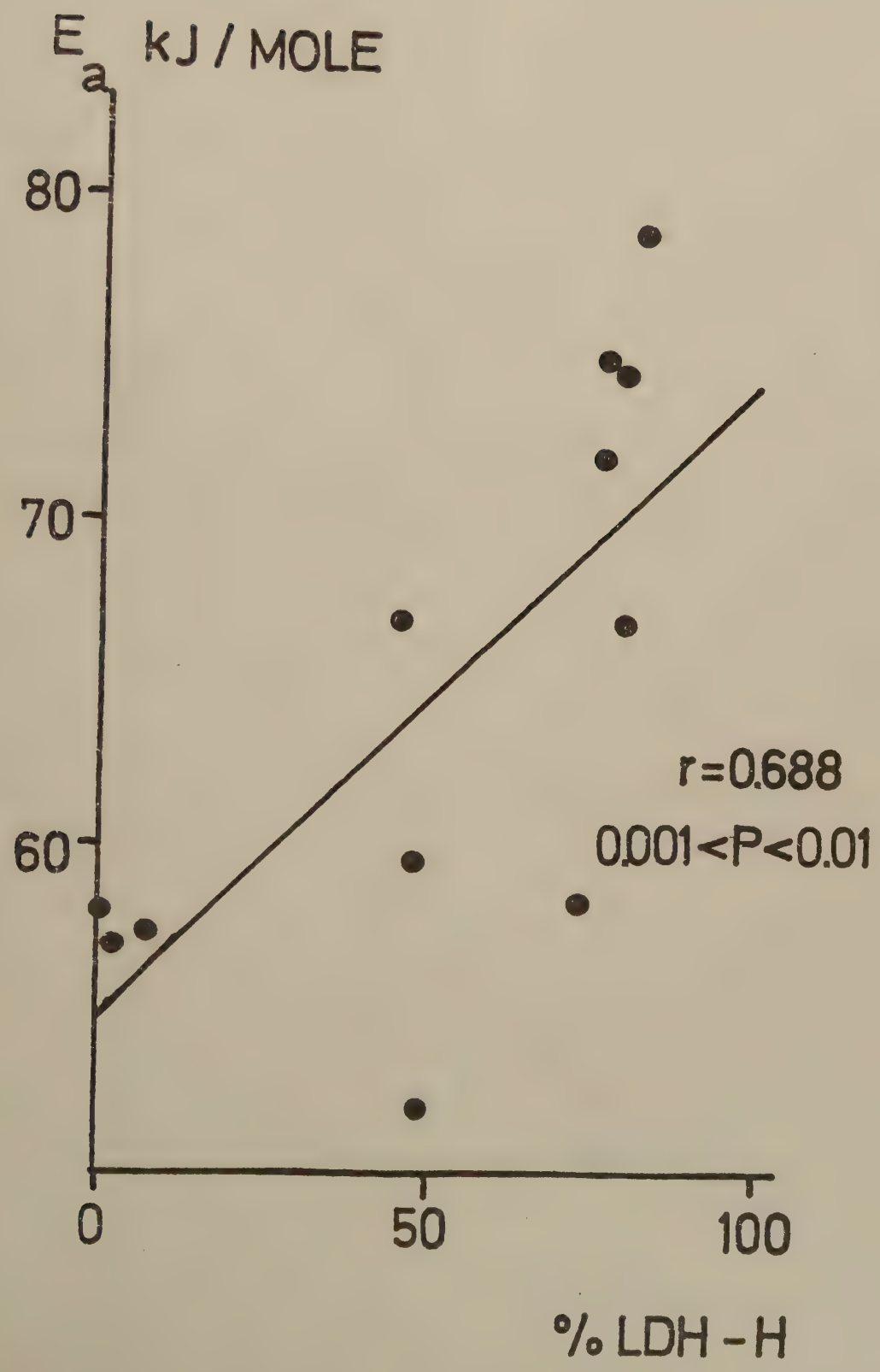


Figure 2

Correlation between the percentage LDH-H (the axis of abscissas) and the energy of inactivation Δ_i of LDH (the axis of ordinates) from the results presented in table 1 and 4.

LDH

ENERGY OF INACTIVATION

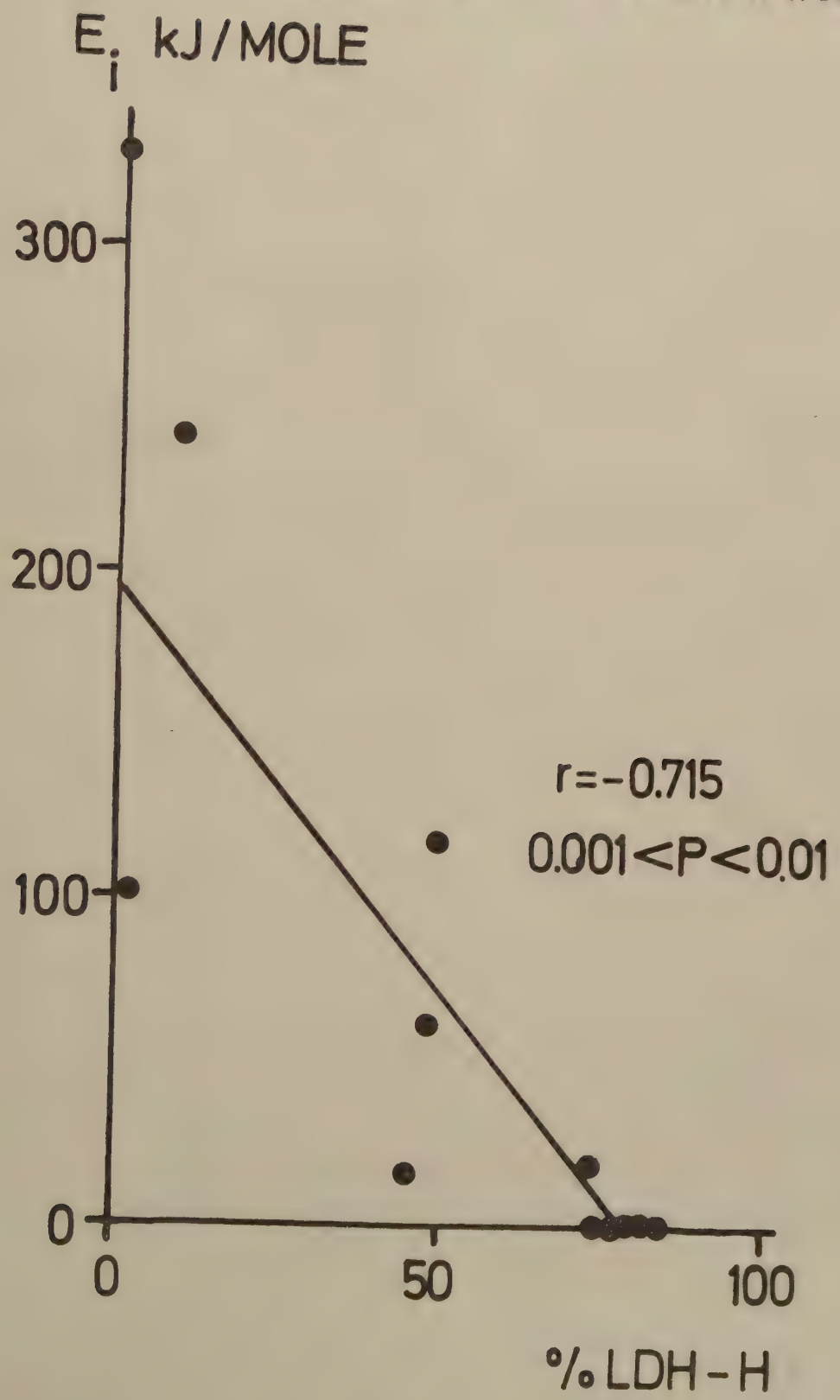


Figure 6

Correlation between the percentage C-MDH (the axis of abscissas) and the energy of activation E_a of MDH (the axis of ordinates) from the results presented in table 2 and 4.

MDH

ENERGY OF ACTIVATION

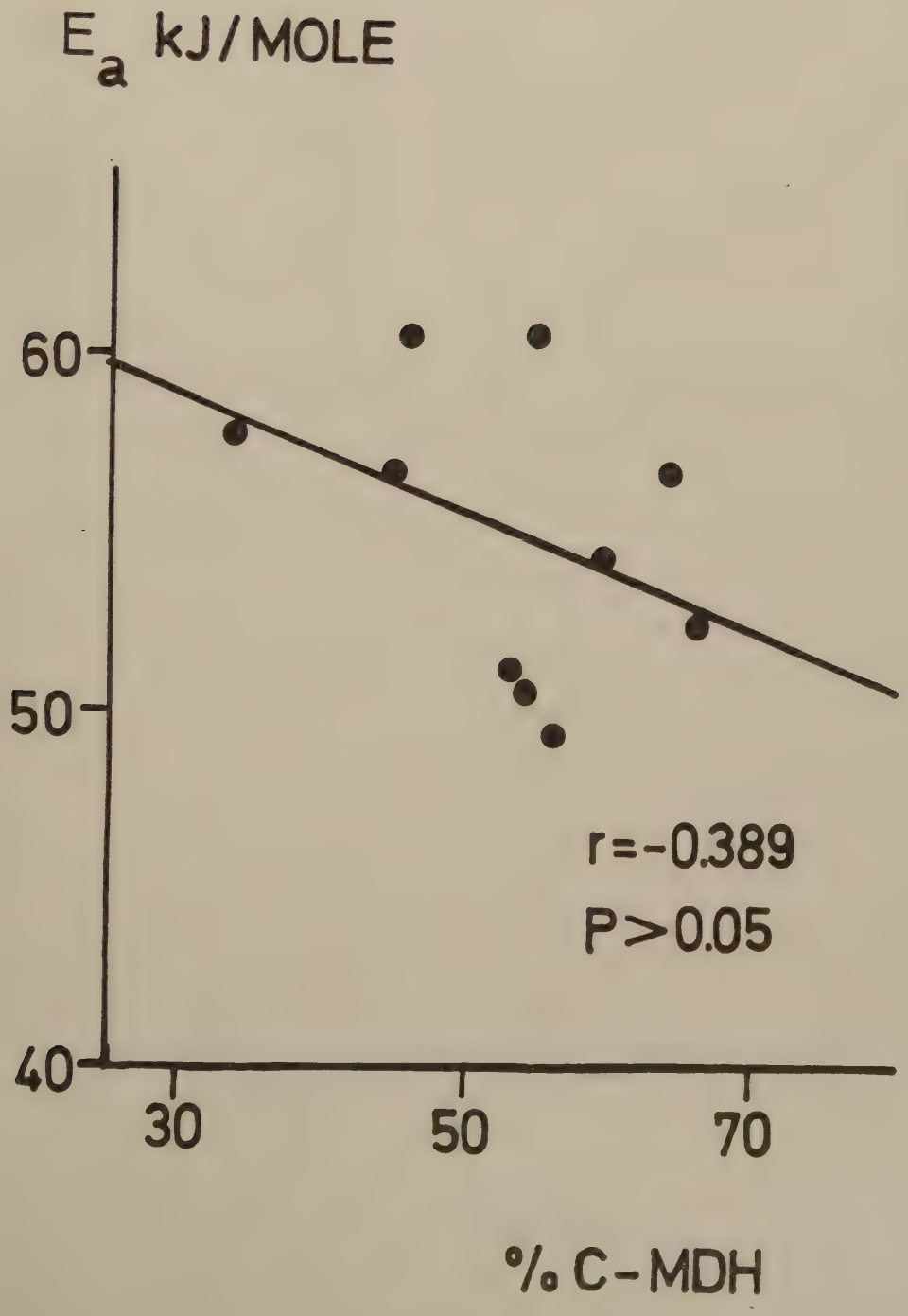


Figure 7

Correlation between the percentage LDH-H (the axis of abscissas) from hedgehog heart and the energy of activation E_a (the axis of ordinates) of hedgehog heart LDH presented in Olsson (1972 a).

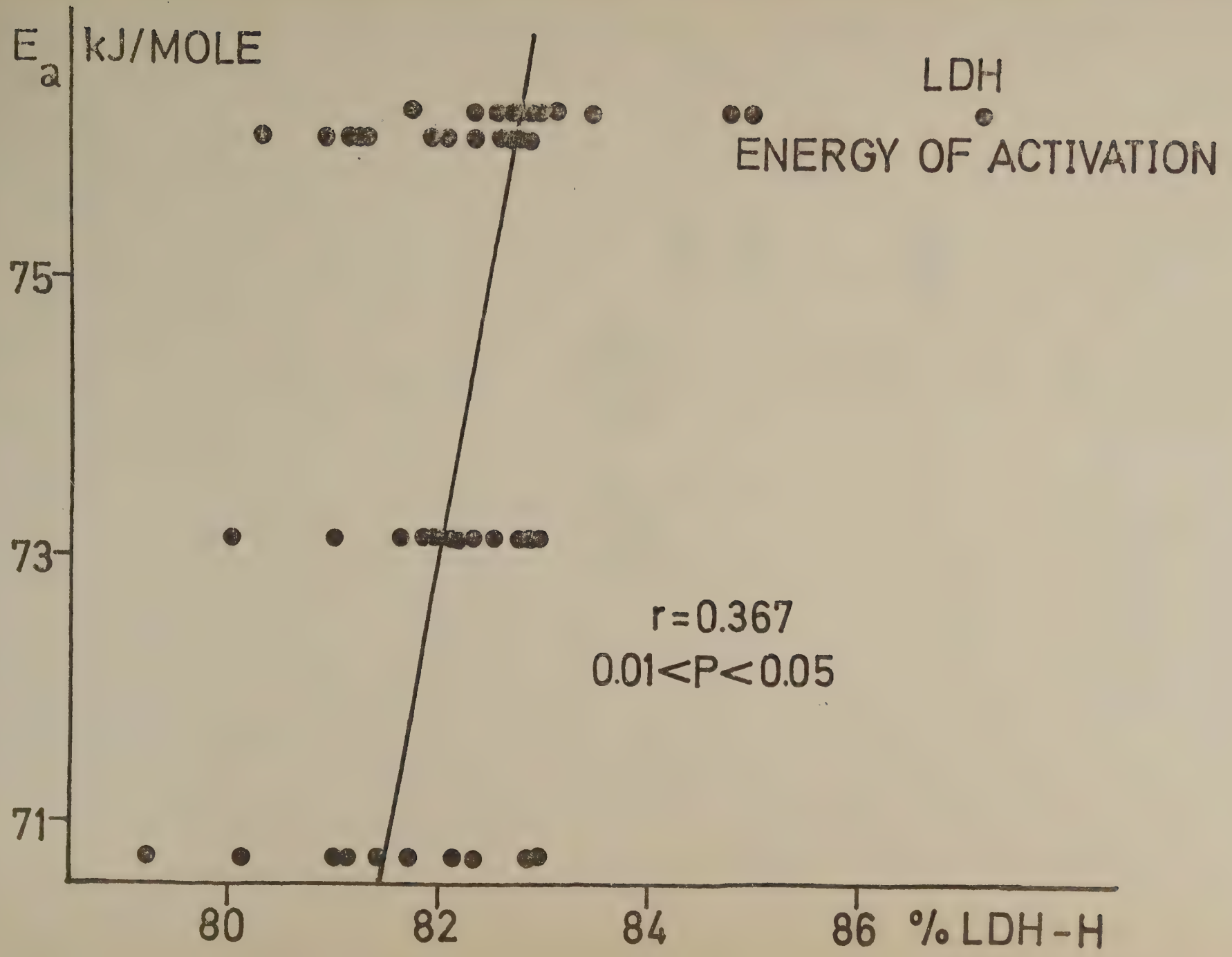
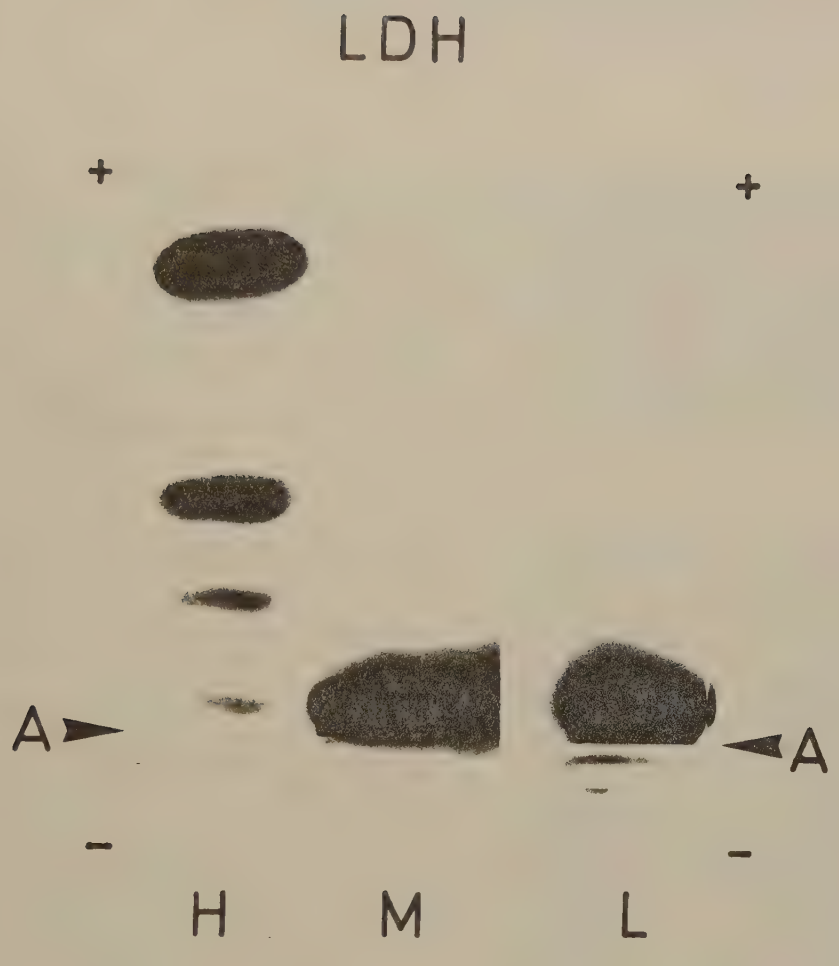

E_a kJ/MOLE
LDH
ENERGY OF ACTIVATION
75
73
71
r=0.367
0.01<P<0.05
80
82
84
86
% LDH-H

FIG 7

Figure 8

Electrophoretic separation of the LDH-isoenzymes from heart (H), skeletal muscle (M) and liver (L) from the frog Rana temporaria. (A) point of application.



Rana temporaria

Figure 9

Electrophoretic separation of the MDH-isoenzymes from (a) guinea pig heart, (b) hedgehog heart, (c) bat heart and (d) frog heart and skeletal muscle, illustrating the different electrophoretic anodal mobilities of cytoplasmatic (C-MDH) MDH. The zymogram (d) is presented with a higher degree of magnification than the other. As a reference, hedgehog heart MDH is separated on the same gel as the frog MDH. (A) point of application.

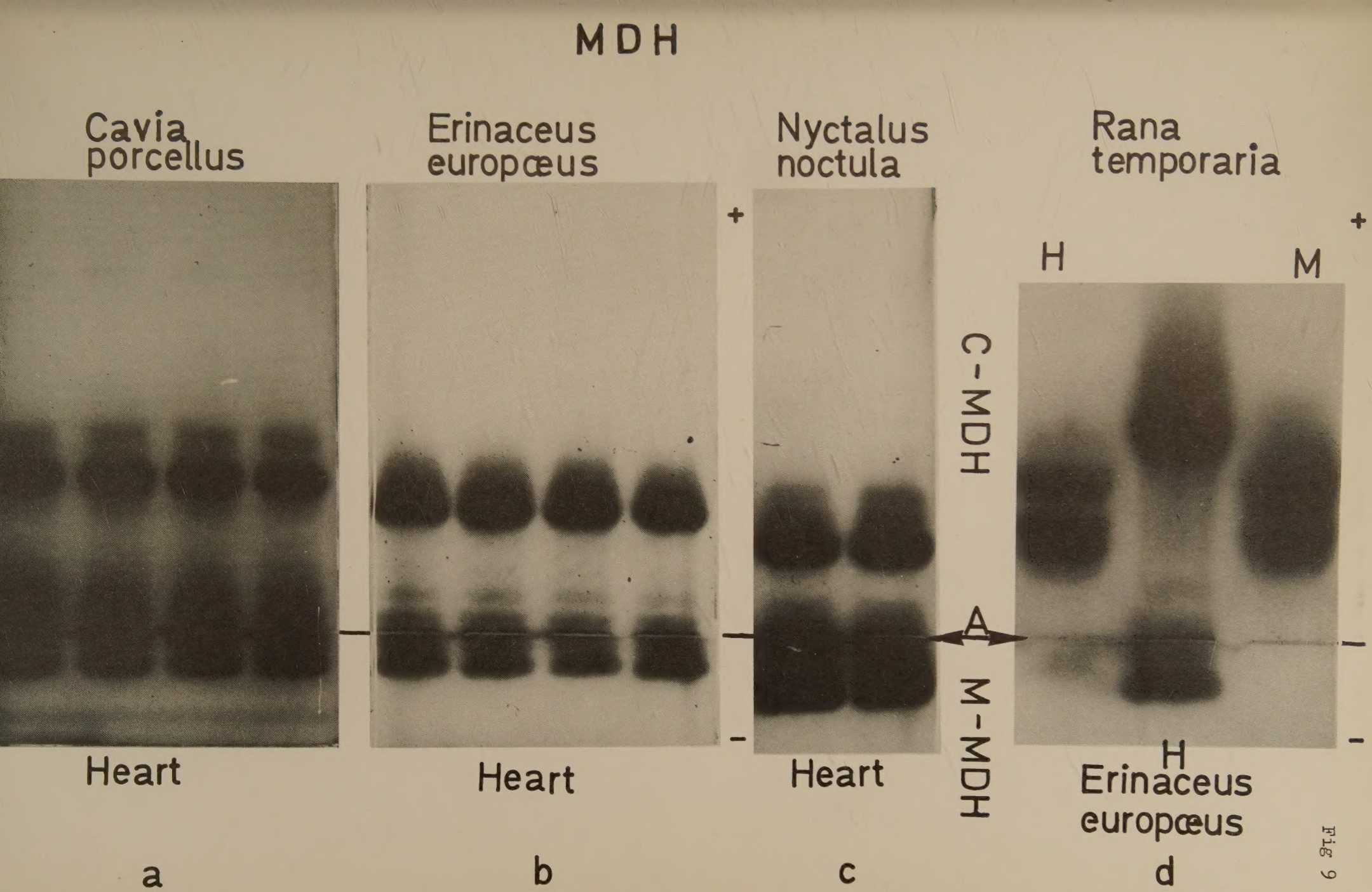

MDH
Cavia porcellus
Erinaceus europœus
Nyctalus noctula
Rana temporaria
+
-
H
M
C-MDH
A
M-MDH
Heart
Heart
Heart
H
Erinaceus europœus
a
b
c
d

Fig 9